STUDIES OF THIAMINE ABSORPTION IN MAN

A Thesis submitted for the Degree

of

Doctor of Philosophy

by

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FOREWORD

Beri-beri has been known for centuries; its relationship to a vitamin deficiency state, subsequently identified as thiamine, has been appreciated since 1885. Although the incidence of beri-beri has greatly decreased, significant numbers of people still develop thiamine depletion syndromes. Thiamine deficiency is a major cause for clinical abnormalities in the malnourished alcoholic (Baker et al., 1964; Leevy et al., 1965a; Leevy et al, 1965b; Fennelly et al., 1964; Fennelly et al., 1967; Thomson, Baker and Leevy, 1968; Leevy and Baker, 1968 and Leevy, Thomson and Baker, 1969) and is frequently present in elderly subjects (Brin et al., 1964; Brin et al., 1965; Griffiths et al., 1965). Knowledge of the site and mechanism of thiamine absorption in animals is still incomplete despite numerous experimental studies and no satisfactory routine test has been developed to measure absorption in man.

It was the purpose of this work to develop a test to measure absorption in man using 35S-thiamine hydrochloride. This provided a method to investigate the route and mechanism of thiamine hydrochloride absorption in man and to study factors which modify it. Special attention was directed to the influence of gastrointestinal disease, age, hepatic steatonecrosis and renal disease. The site of the

absorption defect found in the alcoholic patient was studied and the aetiological roles of ethanol administration and malnutrition were investigated. The utilization of a different mechanism and greater absorption in man incident to changes in the molecular configuration of thiamine (to produce the analogue thiamine propyl disulphide, TPD) were studied and its use in the treatment of thiamine deficiency syndromes was evaluated.

The investigations were begun in the Department of Therapeutics, University of Edinburgh, where I was working under the close supervision of Dr. I. W. Delamore and Professor R. H. Girdwood who first introduced me to the problem of thiamine absorption and guided and encouraged me during the difficult early stages of the investigation. A leave of absence was granted to allow me to continue my work in the Department of Hepatic Metabolism and Nutrition, New Jersey College of Medicine. During this time I was working under the direction of Professor Herman Baker and the head of the department, Professor Carroll M. Leevy. I would like to express my deep gratitude for their invaluable guidance and sincere interest during my graduate training.

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GENERAL INTRODUCTION

Principles of absorption tests in human subjects

Methods for the study of intestinal absorption were reviewed by Verzár and McDougall in 1936. Since then many new techniques have been developed (Levin, 1967). Different approaches have evolved because no single method or parameter is satisfactory for characterizing intestinal absorption and many induce changes which influence the interpretation of the data.

Absorption of a substance by the mucosal cell refers to movement of that substance from the intestinal lumen into the cell and movement from the cell into the subcellular tissue space, blood and lymphatics (Parsons, 1970). This definition implies movement in a particular direction and allows the distinction to be made between influx from the lumen into the mucosal cell and efflux from the cell. It should be recognized that absorption is the net result of two unequal fluxes into and out of the cell at the luminal surface and two similar fluxes occurring across the base of the cell.

The inaccessibility of the gastro-intestinal tract has led to the development of invitro techniques to study intestinal absorption. Unfortunately, however, while providing much valuable information the conditions are very different from those prevailing in the intact animal and the results

require confirmation by invivo experiments. Problems in technique and experimental design meet their greatest difficulty in man. The methods reviewed will be divided into those which assess absorption indirectly by measuring the level of the substance in the blood or excretory products and those which assess it directly in the intestine.

Indirect Methods.

Balance studies The classical method estimates absorption by subtracting the total output of the substance over a period from the total intake. The result of the balance depends upon the combined action of absorption and excretion and cannot be used for substances altered in the large bowel by bacteria. The performance of the test may be difficult because of problems in diet analysis and patient cooperation in regulation of intake and accurate collection of specimens. Also, balance studies give no information on the relative importance of intestinal mobility, alterations in the chemical form of the substance presented to the mucosa and the transfer across the intestine.

Techniques based on recovery from the urine of the absorbed substance or derivatives

McCance and Madders in 1930 introduced a method for measuring the absorption in man of substances which are slowly destroyed in the tissues but readily excreted in the urine. Quantitative data was obtained from the rates of excretion of

the substance in the urine when given orally with that occurring after intravenous injection. From the results obtained, R, the ratio of the sugar utilized per unit of sugar excreted, was obtained. The sugar absorbed during any period was given by

$$A = E(R+1)$$

Where E= sugar excreted during the same time.
Using this method, the authors were able to compare the rates of absorption of xylose, arabinose and rhamose.

Tolerance tests. In 1865, Bence Jones investigated the rapidity of absorption of metals by measuring the presence of lithium in the body fluids and tissues after administration of lithium salts by mouth. Measurements of the test substance in blood or urine following a single oral dose has been taken as an index of the rate of absorption and a guide to the amount absorbed. Such tests have been used for measuring the absorption of substances like glucose, xylose, iron and fat in human subjects (Duthie, 1967). Criticisms of the method were reviewed by Josephs (1958). The principal drawbacks are (1) the results are a combination of effects of absorption and intermedary metabolism. (2) the test provides information on the result of taking only a single dose of the substance.

A quantitative measurement of the rate of entry of an absorbed substance into the body has been presented by Scholer and Code, 1954, for example, who determined the rate of

absorption of deuterium oxide in human subjects. If absorption is occurring at a constant rate A gm. per minute into the body fluids of volume V litres, and c gm. per litre is the concentration in body fluids at a given time t minutes after absorption begins

$$A = Kc + Vdc/dt.$$

where D gm min^{-1} = the removal rate by metabolism and renal excretion. Then,

$$D = Kc$$

and

$$A = Kc(1-exp-Kt/V)^{-1}$$

The substrate concentration in the body fluids can only be taken as a measure of the rate of absorption when the value of the term Kt/V is large (Parsons, 1968). The rate of disappearance from the extracellular fluid after intravenous injection provides a measure of the kinetics of disposal.

Assuming (A=0) the term Kt/V for various values of t can be discovered from the relationship

$$Kt/V = In\ (Co/Ct)$$

where Ct= concentration of the substance at time t.
Co= initial uniform concentration after injection.

Radioactive methods One of the earliest uses of isotopically labeled substances in biological studies gave information on absorption in human subjects. In 1934 Hevesy and Hofer caused

human subjects to drink deuterium oxide (heavy water) and found that it appeared in the urine in 30 minutes. Since this time, radioisotopes have been used to facilitate tolerance testing. In 1953, Schilling introduced a method for measuring the absorption of vitamin B12. The radioactive oral dose was given together with a large non-radioactive parenteral injection of the vitamin which reduced retention of the radioactive vitamin in the body. This method was refined by Brain and Booth (1964) who gave tritium labeled pyridoxine orally together with increasingly large non-radioactive doses of the vitamin in order to determine the loading dose required to ensure minimal retention of radioactivity in the body. This method depends upon the assumptions that the radioactive label is not displaced from the molecule, the loading dose does not alter the normal physiological activity of the intestine and that the radioactive and non-radioactive molecules behave identically.

Total body counting has been used for radioactive substances with - emissions. Attempts have been made to measure iron absorption by this method but difficulties have been encountered in the relative efficiency of counting the radioactive iron which is in the gut and that distributed throughout the body (Pollack et al, 1966).

Direct Methods

Intubation of the intestinal tract. In 1934, Miller and Abbott

introduced a double lumen tube with a terminal balloon into the human intestine and were able to sample from an opening proximal to the balloon. Subsequently, a triple lumen tube was advocated (Abbott and Miller, 1936), which permitted sampling from an area isolated between two balloons. However, difficulties arose because inflation of the balloon often caused colicky abdominal pain with possible alteration of the local circulation and leakage around the balloon could not be excluded.

Blankenhorn, Hirsch and Ahrens (1955) suggested a fine polythene tube (external diametre 2mm) should be allowed to transverse the entire intestine and samples could be taken at varying levels following oral administration of a meal containing a marker substance. In 1961 Schedl and Clifton allowed a tube with a blocked lumen 20-30 cm. along its length to pass from the mouth to the anus. The test substance was perfused from the oral end through an outlet and sampled more distally by a portal in the anal end of the tube. The introduction of a double lumen (Holdsworth and Dawson, 1964) and triple lumen tubes (Cooper et al, 1966) have avoided the necessity of waiting for the tube to pass along the full length of the intestine. Absorption can be calculated from a knowledge of the delivery rate of the infusion pump (R) and an analysis of the inflowing and outflowing materials as follows:-

Let G= concentration of substance being infused.
M= concentration of marker substance.
g= concentration of aspirated test substance.
m= concentration of aspirated marker.

assuming a steady state,

Rate of absorption per minute = RG-vg

where vml/min = rate of passage of fluid leaving past the sampling port.

$V = RM/m$ ml $min.^{-1}$

Rate of absorption = $R(G - gM/m)$ $mgm.min.^{-1}$

These methods have been used to study the absorption of many substances (Duthie, 1967). They measure the disappearance of a substance from the intestinal lumen but not its passage into the subcellular region or blood and lymphatics. A thirty minute period of equilibration is required and it is an open question whether the intestine under these conditions is in a physiological state. These methods also rely on a non-absorbable marker like polyethylene glycol which is assumed to travel down the intestine at the same rate as the test substance. This subject has been fully reviewed by Smyth (1961) and Dawson (1965).

Isolated loops A few experiments have been performed with human small intestine isolated during a surgical procedure. For example, the transport of water and electrolytes (Atwell and Duthie, 1964; Schloerb and Lukert, 1963) and glucose, butyrate, methionine have been studied. (McColl and Nissim, 1965). Unknown factors are the trauma of the operation and the effect of anaesthesia.

Conclusion No single method or parameter is adequate to understand intestinal absorption in man. The choice of methods will depend upon the information that the investigator seeks. Despite all efforts, for many substances, final elucidation of the problem will have to await the development of new techniques.

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CHAPTER I

THIAMINE ABSORPTION IN NORMAL SUBJECTS

Introduction and background

Many different approaches in various animal species have been used to investigate the sites and mechanism of thiamine absorption. The resulting picture is confused and incomplete and much still remains to be done. The methods used to investigate the absorption of thiamine in man have often been inadequate because of the limitations of methodology and no reliable single test has been developed which can be used routinely.

The pattern of absorption of substances from the small intestine depends upon three factors. These are firstly, the mechanism of absorption, whether active or passive; secondly, in the case of active transport, the distribution of this mechanism in different segments of the intestinal mucosa; and thirdly, the relationship between the rate of absorption and the rate of transit along the gut. (Booth et al, 1963).

The site and amount of absorption in animals

This was first investigated by Da Silva and Ivy, 1961, using a chronic Thiry fistula in the jejunum or ileum in the dog and determining thiamine by the fluorometric method described by Burch et al, 1952. In both approximately 30%

of the IOmgm. dose of thiamine was absorbed in one hour. Evidence indicated that under the conditions prevailing in the Thiry fistula destruction of thiamine did not occur at least during the first 30 minutes and they also rejected the possibility of excretion of thiamine into the loop altering the dose. The amount absorbed did not vary significantly from day to day.

These findings are in contrast to those of Polin et al, 1964 who investigated absorption in three week old chicks by adding thiamine-2CI4-hydrochloride to the diet at a concentration of either 4.8 mgm/Kgm. or 112 mgm/Kgm. After four days the animals were sacrificed, the radioactivity in various lengths of intestine counted and thiamine estimated by the thiochrome method (Snell and Snell, 1953). At the lower dose, the concentration in the duodenum was higher than in the upper or middle third of the small intestine. However, the amount found in the animal's crop was not stated, the time interval from the last meal nor the effect of sacrifice on distribution along the intestine determined. At the higher dose, the lowest concentration was found in the duodenum.

The progressive increase in concentration of the vitamin found in sections distal to the duodenum was explained by re-excretion into the intestine via the bile or reversed secretion across the intestine. This conclusion, however, was not supported by measurements of the concentration of

thiamine in the bile nor was there any estimate of the expected accumulation of unabsorbed thiamine at the distal end of the intestine.

The suggestion that the lower intestine may secrete thiamine at high dose levels was first made by Gassmann and Ketz, 1961, who presented evidence suggesting that at high blood concentrations, thiamine can pass from the circulation into the lumen of the intestine. How important this is in terms of the net absorption may be ascertained by giving radioactive thiamine parenterally and determining the amount appearing in the faeces. Middleton and Morrison, 1962, working with weanling Winster rats gave doses of 5 µgm., 10 µgm., and 20 µgm. of 35S-thiamine subcutaneously and found 1.3± 0.5%; 1.6± 0.3% and 2.0± 0.3% respectively during the succeeding four days. Iacono and Johnson, 1957, injected 1-10 mgm. intraperitoneally into Sprague Dawley rats and recovered only 0.8% - 1.6% in the faeces in 24 hours. Similar results were found by McCarthy et al., 1954 and Draper, 1958 who recovered 0.3% - 1.4% when 120 µgm. of 35S-thiamine was given intraperitoneally to six young rats. These findings were shown to resemble those found in man where the faecal excretion was measured for seven days after an intravenous injection of 20 mgm. of thiamine and the faecal levels of thiamine were found to be hardly affected (Shinazoid and Katsura, 1965). The blood levels following an intravenous dose will be far higher than after a comparable

oral dose and consequently you might expect the passage into the intestine to be greater.

The site of thiamine absorption was investigated in Wister strain of rats by Middleton and Grice, 1964. In the first experiments, rats weighing 200-250 gm. were given 20 µgm. of 35S-thiamine by stomach tube. The animals were anaesthetised with ether prior to exsanguination at intervals after the oral dose up to 24 hours. At laparotomy, ligatures were tied around the lower end of the oesophagus, the duodenum near the pylorus and the ileum near the ileocaecal junction. The small intestine was divided into four approximately equal ligated segments. The percentage of the oral dose found in a particular area was measured and the total amount absorbed prior to sacrifice, was determined by subtracting the amount remaining in the intestine from the dose given. Within the first half hour, thiamine was found to be present throughout the intestinal tract and 32.9% of the oral dose had already been absorbed. During the second hour, a slight increase in the percentage of radioactivity present in the lower ileum was noted while significant decreases occurred in the more proximal portions. 71% of the administered thiamine had been absorbed at the end of two hours, and 88% after six hours when only very small amounts could be removed from the large intestine. In a second series of experiments, it was shown that removal of up to 70 cm. of ileum did not influence the absorption of thiamine as shown

by faecal analysis.

Consequently, work in different animal species suggests that thiamine absorption can occur throughout the ileum but is maximal in the proximal small intestine. The amount of destruction in this region before most of the thiamine has been absorbed is uncertain and the importance of re-excretion into the lower ileum occurring at physiological dose levels remains in doubt.

The site and route of absorption in man

That thiamine absorption occurs primarily in the upper small intestine in man has been inferred from the rapid rate of absorption. The findings of Bean et al, 1951, in two cirrhotic subjects with unusual connections between the portal vein and a superficial abdominal vein, however, tend to confirm this conclusion and suggest that thiamine is probably absorbed via the portal system. Using the thiochrome method they simultaneously measured the serum levels in the 'portal vein' and the systemic venous circulation following a 5.0mgm. oral dose of thiamine. A number of unanswered questions leave doubts as to the correct interpretation of the data. These include the fact that the communication was only confirmed in one patient, the assumption that thiamine is in a form both in the portal vein and after passage through the liver which can be measured by the thiochrome method and that the absorption in patients with

cirrhosis is the same as in the normal individual. However, the results suggest that absorption is maximal during the first three hours and that this occurs via the portal system.

The influence of gastric secretion on thiamine absorption

It has been suggested that thiamine deficiency may produce achlorhydria which subsequently interferes with absorption of thiamine. However, investigations by Shay et al, 1946, in rats and Gildea et al, 1930; Elson, 1935 and Rafsky et al, 1947 in man failed to demonstrate abnormalities in gastric secretion in thiamine deficiency or any correlation between achlorhydria and reduced thiamine absorption. Wang and Harris, 1939 and Brummer and Markkanen, 1960 measuring daily urinary excretion of dietary thiamine have found reduced excretion in achlorhydric subjects. The influence of achlorhydria on thiamine absorption warrants further investigation using radioisotopes.

Absorption from the large intestine

Less than 2% of physiological doses of thiamine hydrochloride given by enema is absorbed in man (Alexander and Landwehr, 1946; Campbell and Morrison, 1963). These conclusions are supported by work in rats by Middleton and Grice, 1964. Little absorption seems to occur from the large intestine even when large doses are given. The fate of small amounts remaining from a physiological oral dose after passage through the small intestine has not been

directly investigated and the importance of breakdown of thiamine by large bowel bacteria prior to absorption is not known.

The evidence suggests that thiamine absorption is probably maximal in the upper small intestine but that absorption occurs throughout the small intestine. The influence of achlorhydria on thiamine absorption requires further investigation and the degree of breakdown of thiamine by intestinal bacteria prior to absorption has not been estimated in man. The absorption occurring in the large bowel following an oral dose seems to be very limited and probably plays little part under normal conditions.

General Materials and Methods

Radioactive material. All of the thiamine preparations were supplied as the hydrochloride except where it is specifically stated that thiamine propyl disulphide was used. 35S-thiamine was obtained from the Radiochemical Centre, Amersham. The radioactive material used orally had a specific activity of 177 mc/ gm. and was found to be radiochemically pure when tested in the three chromatographic systems described below. The solid material was dissolved in distilled water, 100 μc (10 doses) dispensed into each ampule, freeze dried, and stored at -20°C. It was reconstituted by adding 10 ml of distilled water. In a few experiments, radioactive thiamine was used intravenously. This material

was supplied in aqueous solution pH 5; it had a specific activity of 12.0 μc/ml. and analysis showed it was more than 90% radiochemically pure. The non-radioactive thiamine was supplied in ampules containing 100 mgm. in one ml. by Roche Products Ltd., England.

Method for the study of absorption of 35S-labelled thiamine. The radioactive material was diluted with non-radioactive thiamine so that each test dose contained from 1.0 mgm. to 200 mgm. and 10 μc of radioactivity dissolved in 20 ml. of water. A quantity of the solution from which the test dose was prepared was used as a standard. The test dose was given orally to subjects after an overnight fast, and in some experiments a parenteral injection of non-radioactive thiamine was given immediately before the oral dose. The standard test referred to later includes both a 1.0 mgm. oral dose of radioactive thiamine and a parenteral injection of 200 mgm. non-radioactive thiamine given at the same time as the oral dose. Absorption was assessed by measuring the urinary radioactivity during the following 24 to 72 hours. Arterial blood was collected via a Cournard needle in vacutainers^{R} at 0, 3, 6, 10, 20, 30, 40, 60, 90, 120, 150 and 180 minutes or more frequently. Urine was collected hourly for the first five hours and then after 12, 24, 48 and 72 hours. Samples were counted in a Packard Tricarb Scintillation counter using 0.8 gm. per cent of 2-5 diphenyloxazole and 0.005 gm. per cent of 1,4-bis(2(phenyloxazolyl)) benzene

dissolved in toluene as the liquid scintillator. The samples were prepared by adding 1.0 ml. of serum or urine to 3 ml. hyamine chloride, 1.5M solution in methanol, and adding 10ml liquid scintillator. 10μc of the test dose was added as an internal standard to each sample.

Accuracy of counting of radioactivity in urine. Known amounts of 35S-thiamine were added to three equal volumes of urine to give final thiamine concentrations similar to those encountered in the tests on subjects. The urines contained approximately 8.0 nc/ml, 0.8 nc/ml. and 0.04 nc/ml. The urines were shaken and twenty samples taken from each (only ten from 0.04 nc/ml. urine) using the same pipette which was washed between samples. Liquid scintillator was added by pipette and each sample was counted twice. The results are shown in Tables 1a, 1b, and 1c. It will be seen that variation due to sample preparation and counting is extremely low and well within the experimental error introduced by other factors.

Modified extraction procedure for urinary radioactivity. The method used was the phenol extraction procedure of Iacono and Johnson, 1957 modified following the experiments described later in this chapter, page 65. The inorganic salts were first removed by the phenol extraction procedure of Crammer, 1948, as these were known to cause streaking and tailing of radiometabolites on chromatograms with the various solvents

Table 1a. Duplicate counts of twenty specimens prepared from the same urine sample

a. At the concentration of 8.3 nc*/ml.

Sample No.	1st time counted nc/ml	2nd time counted nc/ml
1	8.592	8.473
2	8.363	8.511
3	8.316	8.271
4	8.269	8.338
5	8.078	8.277
6	8.134	8.281
7	8.506	8.485
8	8.539	8.395
9	8.201	8.123
10	8.409	8.447
11	8.220	8.057
12	8.201	8.207
13	8.258	8.245
14	8.308	8.173
15	8.322	8.335
16	8.265	8.086
17	8.320	8.277
18	8.231	8.325
19	8.346	8.447
20	8.332	8.306

$\Sigma x = 166.240$

$\bar{x}$ 8.312

$\Sigma x^2 = 1382.10338$

$(\Sigma x)^2 = 27635.74$

$\frac{(\Sigma x)^2}{20} = 1381.787$

$\sigma_x = .13$

$8.3 \pm .1$ nc/ml

$\Sigma y = 166.06$

$\bar{y}$ 8.303

$\Sigma y^2 = 1379.1421$

$(\Sigma y)^2 = 27576.26$

$\frac{(\Sigma y)^2}{20} = 1378.813$

$\sigma_y = .13$

$8.3 \pm .1$ nc/ml

*nc = nanocurries

Table 1b. Duplicate counts of twenty specimens prepared from the same urine sample

b. At the concentration of 0.76 nc/ml.

Sample No.	1st time counted nc/ml	2nd time counted nc/ml
1	.800	.788
2	.780	.769
3	.774	.760
4	.759	.769
5	.753	.760
6	.784	.772
7	.751	.767
8	.769	.758
9	.757	.748
10	.756	.756
11	.775	.765
12	.766	.776
13	.770	.761
14	.773	.770
15	.755	.761
16	.780	.788
17	.766	.765
18	.773	.765
19	.757	.749
20	.772	.758

$\Sigma x = 15.370$

$\bar{x} = .7685$

$\Sigma x^2 = 11.81472$

$(\Sigma x)^2 = 236.2369$

$\frac{(\Sigma x)^2}{20} = 11.811895$

$\sigma_x = \Sigma x^2 - \frac{(\Sigma x)^2}{20} = .00283$

$.76 \pm .1$ nc/ml

$\Sigma y = 15.305$

$\bar{y} = .7653$

$\Sigma y^2 = 11.714245$

$(\Sigma y)^2 = 234.2430$

$\frac{(\Sigma y)^2}{20} = 11.71215$

$\sigma_y = .00209$

$.76 \pm .1$ nc/ml

nc = nanocurries

Table 1c. Duplicate counts of ten specimens prepared from the same urine sample

c. At the concentration of 0.04 nc/ml.

Sample No.	1st time counted nc/ml	2nd time counted nc/ml
1	.0417	.0414
2	.0422	.0410
3	.0394	.0413
4	.0411	.0415
5	.0406	.0399
6	.0403	.0393
7	.0408	.0396
8	.0410	.0403
9	.0416	.0411
10	.0393	.0401

$\Sigma x = .4080$

$\bar{x} = .0408$

$\Sigma x^2 = .0166544$

$(\Sigma x)^2 = .166464$

$\frac{\Sigma x^2}{10} = .0166464$

$\Sigma x^2 - \frac{\Sigma x^2}{10} = .0166544 - .0166464 = .0000080$

$S.D. = \sqrt{\frac{.000008}{9}}$

$.00028$

$.0408 \pm .00028$ nc/ml

$\Sigma y = .4055$

$\bar{y} = .0406$

$\Sigma y^2 = .01645288$

$(\Sigma y)^2 = .1644302$

$\frac{\Sigma y^2}{10} = .01644302$

$\Sigma y^2 - \frac{\Sigma y^2}{10} = .0164528 - .0164430 = .0000098$

$S.D. = \sqrt{\frac{.00000980}{9}}$

$.00030$

$.0406 \pm .0003$ nc/ml

nc= nanocurries

employed. The extraction was carried out in a separating funnel by adding an equal volume of water-saturated phenol to the urine, the mixture shaken vigorously, and allowed to separate overnight at 4°C. The phenol layer was then drained off and washed several times with water to remove the last traces of salt. Twice the volume of diethyl ether and water (40:1) was then added to the phenol extract and the mixture shaken. Within minutes, the water phase containing the metabolites settled to the bottom of the funnel and was drained off. The ether-phenol mixture was washed several times with small volumes of water and the washings added to the original extract.

In order to eliminate still more of the urinary solids and yet retain the radioactivity in solution, the urinary extract was concentrated in vacuo at 35°C. to about 3 ml. Approximately 35 ml. of absolute ethanol was added and the volume again concentrated to four ml. The resulting precipitate did not contain any radioactivity and was discarded. The method was consistently found to remove approximately 90% of the radioactivity present in the original sample.

Descending chromatography was performed on Whatman no.1 paper using N-propanol-water-IM acetate buffer pH5 (70:20:10), the upper phase of sec-butanol/water as solvents. The papers were scanned in a BTL Radioactive Chromatogram Scanner and radioautographs were prepared.

Radioautographs. For the preparation of autoradiographs, the dried paper chromatograms were marked in all four corners with radioactive ink and then placed in contact with Gavert non-screen X-ray film in a 14 x 17 in. Wolf-Kirshner x-ray film exposure holder and placed under a press in the dark; the film was exposed to the chromatogram for up to three months, depending upon the amount of radioactivity. The films were developed in a conventional manner using Kodak developing facilities.

Identification of Thiamine Compounds on Paper Chromatograms.

A. Thiochrome test. After drying the chromatograms at room temperature thiamine and its esters present on the paper were converted to the corresponding thiochromes by the method of Siliprandi & Siliprandi, 1954; this test will detect 1.0µgm. thiamine.

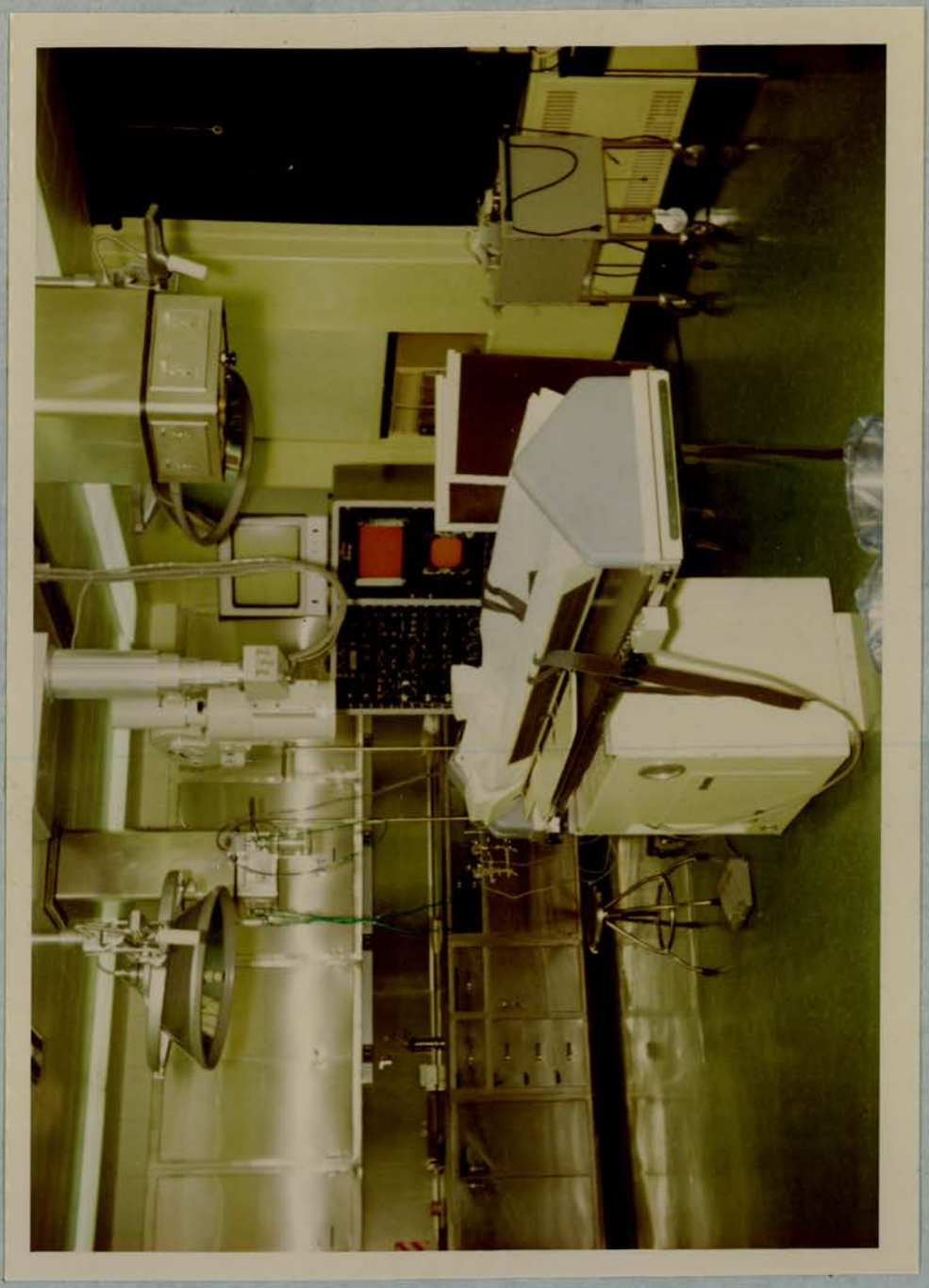
B. Bioautography. was used to check the biological activity of the thiamine extracted from the urine and small intestinal juice. Some chromatograms were not sprayed but were scanned and the radioactivity eluted with 3 ml.distilled water. The ability of the eluate to support the growth of O.danica, which requires intact thiamine (Heinrich, 1955; Baker, et al, 1964), was compared with a control consisting of an equivalent area cut from the same chromatogram. The method of Baker etal, 1964, was used for thiamine assay. Following incubation, the material was centrifuged at 3,000

revolutions per minute for 30 minutes and subsequently washed by centrifuging after resuspension in two aliquots of 20ml. sterile distilled water. The radioactivity could not be washed from the organisms. The organisms were finally suspended in gel-scintillator and the radioactivity present counted by well-scintillation counting.

C. Separation of radioactive compounds. To confirm that complete separation had been achieved, two chromatograms were run in each of the three solvent systems and the radioactivity corresponding to thiamine eluted as above. Each of the elutes was then re-run in a different chromatographic solvent system and produced a single spot at the same Rf as thiamine. The radioautographs also showed a single discrete spot.

Hepatic vein catheterization Catheterization of the hepatic vein was carried out according to the method of Leevy and Gleidman, 1958 using the facilities shown in Figure 1. Studies were performed in the basal state. With sterile precautions, a right or left basilic vein was exposed, and the largest catheter accepted by the vein, connected with a three-way stopcock, was inserted and advanced to the right atrium. A constant slow drip of saline solution containing 50 mgm.of heparin per litre was allowed to flow through the catheter to prevent intraluminal clotting. The catheter was introduced under fluoroscopic

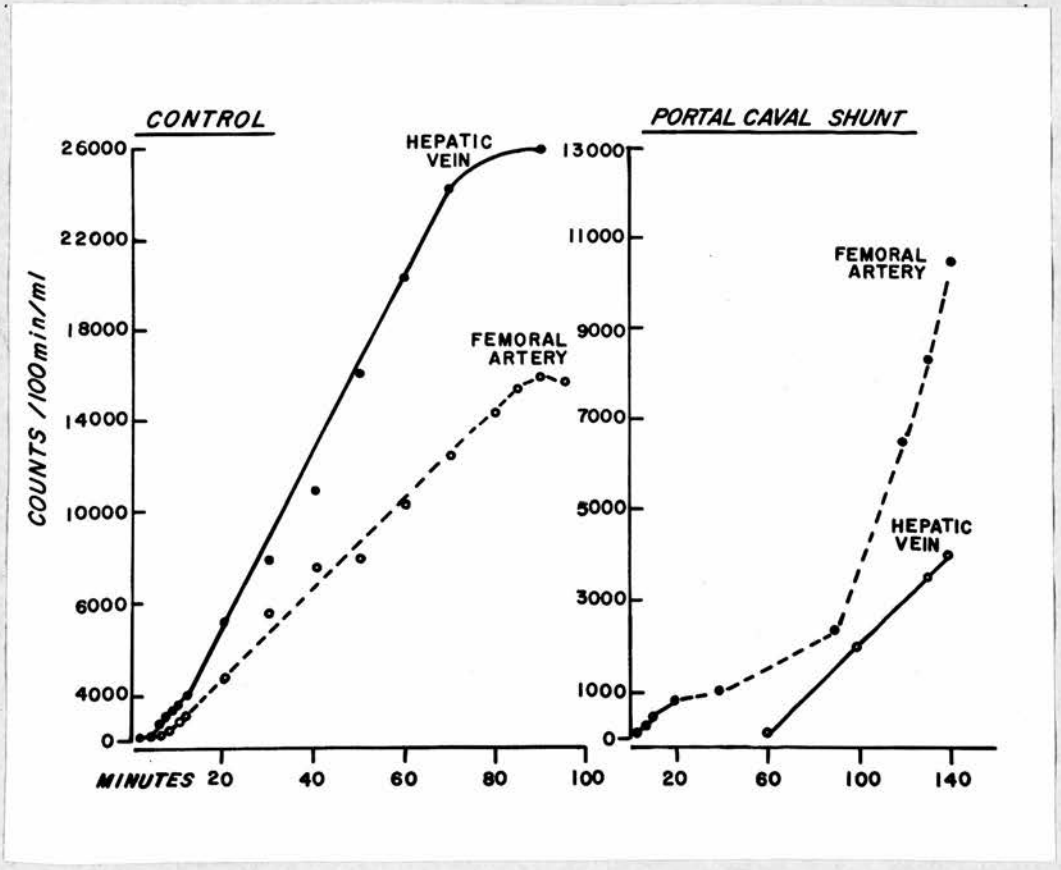
FIGURE 1. Catheterization Room



control into the inferior vena cava by being turned so that the curved tip pointed right and anteriorly. The tip was guided into the right hepatic vein and advanced to the wedged position in the small hepatic venule. The position of the tip of the catheter was confirmed by observation of the pressure curve and the injection of contrast medium. Withdrawal to the 'free' position permitted blood sampling from the hepatic vein. A Cournand indwelling arterial needle was introduced into the femoral artery. Hepatic blood flow was determined by the Fick principle using indocyanine green (ICG) (Leevy et al., 1962).

Umbilical vein catheterization was performed using the method of Lavoisie et al., 1966 by a surgeon in the operating theatre. Under local anaesthesia, a 5 cm. long cutaneous incision was made half way between the xyphoid process and the umbilicus. After dissection of the two layers of the falciform ligament, the round ligament was identified, laying on the peritoneum. It was then opened and dilated with a Bakes dilator. A membrane closes the former umbilical vein at the portal junction and, when punctured, allowed portal blood to flow back into the umbilical vein. A ureteral catheter (size 8, nylon) was then placed via the umbilical vein, into the right branch of the portal vein. The position of the catheter is confirmed by the injection of radio-opaque dye (Figure 2). A constant slow

FIGURE 2. Hepatogram obtained through transumbilical catheterization with the end of the catheter in the right portal vein.


CONTROL
PORTAL CAVAL SHUNT
COUNTS /100min/ml
26000
22000
18000
14000
10000
6000
4000
0
HEPATIC VEIN
FEMORAL ARTERY
MINUTES 20 40 60 80 100
13000
11000
9000
7000
5000
3000
1000
0
FEMORAL ARTERY
HEPATIC VEIN
20 60 100 140

drip of normal saline containing 50 mgm.of heparin per litre was allowed to flow through the catheter to prevent intraluminal clotting.

RESULTS

Investigation of test conditions. The 24 hour excretion of radioactivity when ten control subjects were only given 1.0 mgm.of radioactive thiamine orally was 6.1% (SEM ± 0.64) but if the oral dose was preceded by giving 300 mgm. of non-radioactive thiamine intravenously 48 hours before the excretion of radioactive thiamine rose to 15.3% (SEM ± 2.63). This difference is highly significant. ($t = 4.26$, $p<0.01$). (Table 2).

The standard test. The effect of varying the size of an intravenous dose of non-radioactive thiamine given at the same time as the oral dose of radioactive material was studied and the optimum amount for this intravenous flushing dose was found to be 200 mgm. This dose ensured maximal excretion and will be referred to as the standard test of absorption in this section (Table 3; Figure 3). Giving thiamine to control subjects two days before they received the standard test did not alter the mean urinary excretion of radioactivity (Table 4). ($t = 0.814$; $0.2 < p < 0.5$).

The duration of the flushing dose

The duration of the flushing dose was investigated in

Table 2. Excretion of radioactivity (%) after 1.0 mgm. 35S-thiamine hydrocholoride orally before and after *saturation in controls

Before Saturation		After Saturation	
	3.8		4.9
	4.1		12.1
	4.3		14.0
	4.5		17.5
	4.7		21.5
	7.2		22.0
	7.5		
	7.5		
	7.8		
	9.6		
MEAN	6.1	MEAN	15.3
S.E.	0.64	S.E.	2.63

* Subjects saturated with 300 mgm. thiamine hydrochloride i.v. 48 hours before test.

FIGURE 3. Twenty-four hour urinary excretion of radioactivity after 10 mgm. 35S-thiamine dydrochloride (THCl) orally with varying amounts of non-radioactive thiamine hydrochloride intravenously in control subjects.

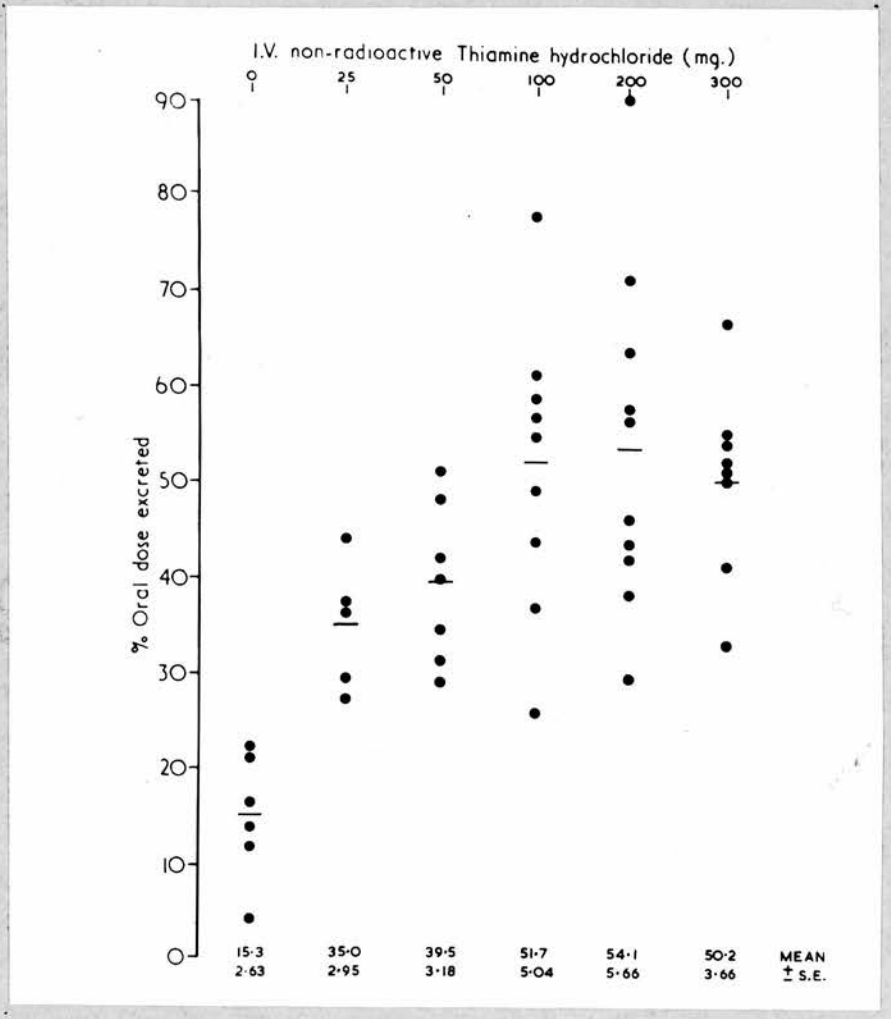

I.V. non-radioactive Thiamine hydrochloride (mg.)
0
25
50
100
200
300
% Oral dose excreted
90
80
70
60
50
40
30
20
10
0
15·3
2·63
35·0
2·95
39·5
3·18
51·7
5·04
54·1
5·66
50·2
3·66
MEAN
± S.E.

Table 3. Twenty-four hour urinary excretion of radioactive thiamine by normal subjects

The effect of injecting varying amounts of non-radioactive thiamine at the time of administering 1.0 mgm. radioactive thiamine by mouth on the excretion of radioactive thiamine in the urine.

Intravenous dose of non-radioactive thiamine	Urinary excretion of radioactivity %oral dose*	
mgm.	Before loading	After loading**
0	6.1 ± 0.64 (10)	15.3 ± 2.63 (6)
25		35.0 ± 2.95 (5)
50		39.5 ± 3.18 (7)
100		51.7 ± 5.04 (9)
200	48.2 ± 4.54 (10)	54.1 ± 5.66 (10)
300		50.2 ± 3.66 (8)

*The results are expressed as the mean - SEM (n) Urine was collected for 24 hours.

**Subjects given 300 mgm. thiamine i.v. 48 hours before test, and a further dose of thiamine at the time of the test.

Table 4. Effect of saturation on results of standard oral test* in control subjects

Before Saturation	After Saturation
32.9	29.7
35.8	38.8
39.2	43.6
42.7	47.1
43.2	56.6
43.9	58.1
46.5	63.5
52.7	71.0
63.0	90.7
81.6	
MEAN 48.2	MEAN 54.1
S.E. 4.54	S.E. 5.66

*Standard Oral Test = 1.0 mgm. thiamine - 35S HCl orally 200 mgm. thiamine hydrochloride intravenously. Saturated as before, see Table 3.

six subjects by incorporating radioactive thiamine (5.0μc) in the 200 mgm. parental injection of non-radioactive thiamine. The results following intravenous and intramuscular administration are shown in Figures 4, 5, and 6. The rate of disappearance from the serum was not exponential but a straight line relationship could be obtained if the serum level was plotted against log(log) time.

Eighty-eight per cent of the intravenous dose was recovered during the first 24 hours and 90% after the radioactive thiamine was given intramuscularly to the same subject. (Figures 4 and 5). Four other subjects excreted more than 93% of an intravenous dose in 24 hours. Consequently, it seems that practically all of the oral dose absorbed, once it reaches the blood stream, will probably be excreted during the first 24 hours, under the conditions of the standard test.

Adequacy of the flushing dose

The adequacy of the 200 mgm. flushing dose was tested further by varying the time of the flushing dose or maintaining a high blood level of non-radioactive thiamine during the first 16 hours by repeated injections but this had a negligible effect on the total urinary recovery (Table 5). When the standard test alone was repeated in four subjects, the following results were obtained:

FIGURE 4. The radioactivity in serum and urine after administration of radioactive thiamine hydrochloride (THCl) intravenously along with 200 mgm. non-radioactive thiamine hydrochloride.

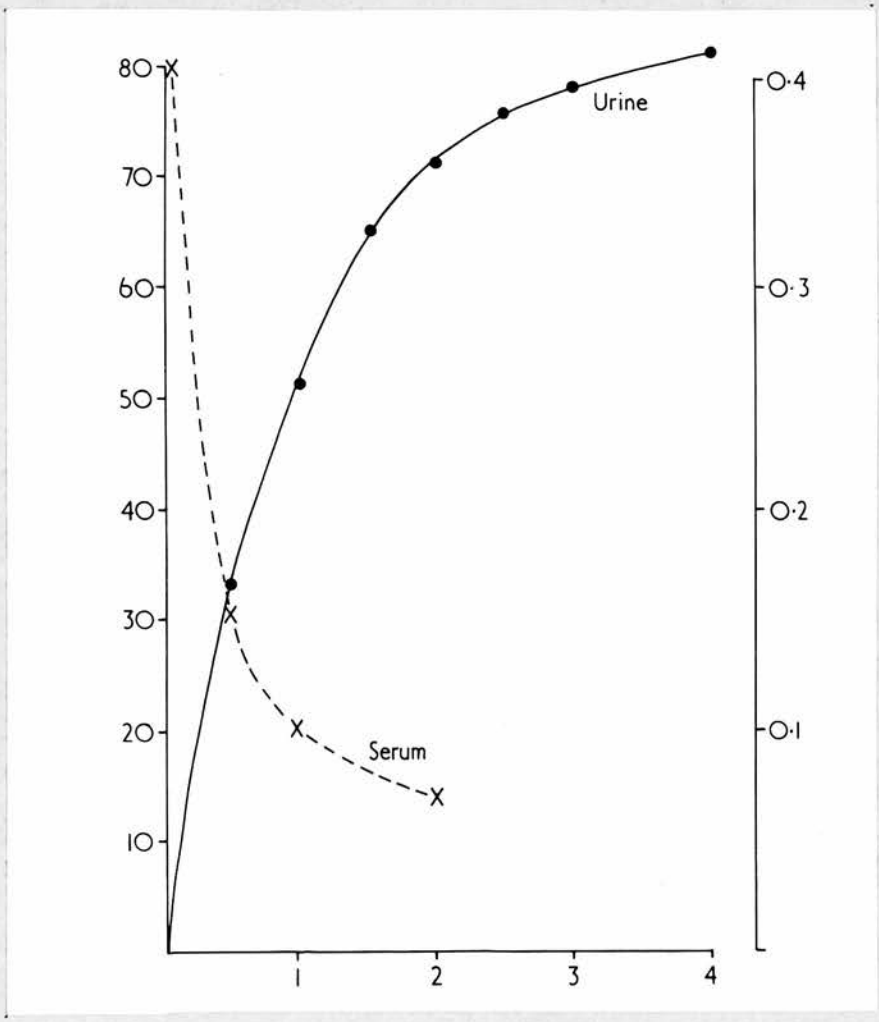

% INTRAVENOUS 35S-THIAMINE HCl EXCRETED
80
70
60
50
40
30
20
10
Urine
Serum
0·4
0·3
0·2
0·1
SERUM LEVEL NANOCURRIES/ML
1
2
3
4
HOURS

FIGURE 5. The radioactivity in serum and urine after administration of radioactive thiamine hydrochloride (THCl) intramuscularly on a different occasion to the same control subject as in figure 4. 200 mgm. of non-radioactive thiamine hydrochloride was given along with the radioactive thiamine.

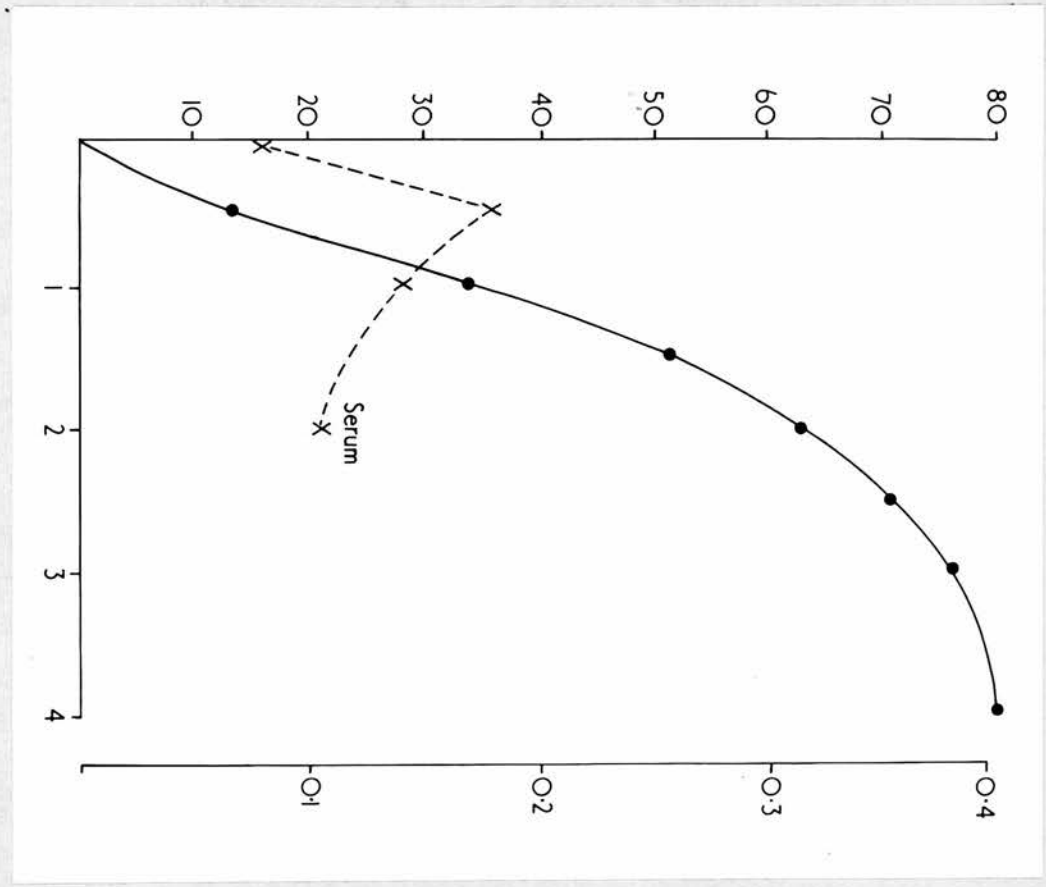

% INTRAMUSCULAR 35S-THIAMINE HCl EXCRETED
10
20
30
40
50
60
70
80
Serum
1
2
3
4
HOURS
0·1
0·2
0·3
0·4
SERUM LEVEL NANOCURRIES/ML

FIGURE 6. The rate of removal of radioactivity from the serum after 5.0 mgm. of 35S-thiamine hydrochloride (THCl) and a 200 mgm. of non-radioactive thiamine hydrochloride in a control subject and a patient with reduced absorption.

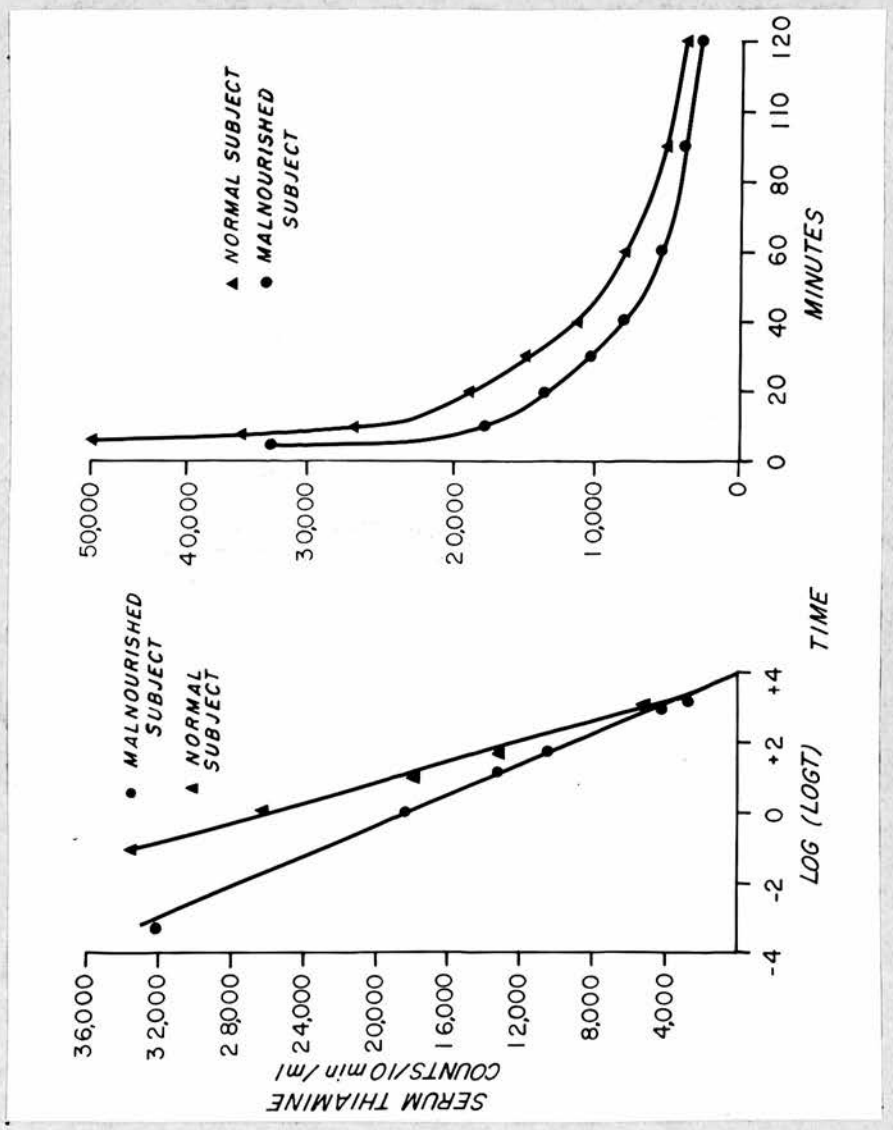

SERUM THIAMINE
COUNTS/10 min /ml
36,000
32,000
28,000
24,000
20,000
16,000
12,000
8,000
4,000
-4
-2
0
+2
+4
LOG (LOGT)
TIME
MALNOURISHED SUBJECT
NORMAL SUBJECT
50,000
40,000
30,000
20,000
10,000
0
0
20
40
60
80
110
120
MINUTES
NORMAL SUBJECT
MALNOURISHED SUBJECT

Table 5. Effect of varying the time of the initial flushing dose and giving additional intravenous flushing doses

Subjects were given two tests: First* and Second** and received 300 mgm. non-radioactive thiamine intravenously 48 hours before each test.

Subject	Test	^{35}S-thiamine urinary excretion %
1	First	49.0
	Second	53.0
2	First	49.0
	Second	49.0
3	First	41.7
	Second	41.2
4	First	75.0
	Second	80.0
5	First	58.5
	Second	58.0
6	First	59.9
	Second	59.0

*First test = 1.0 mgm. radioactive thiamine orally and 200 mgm. non-radioactive thiamine flushing dose intravenously at the same time.

**Second test = Subjects 1-3 same as first test with additional 100 mgm. flushing doses at 2, 6 and 12 hours after oral dose.

4: - same as first test with additional 200 mgm. at 4 hours.

5: - received 200 mgm. i.m. only at time of oral dose.

6: - no flushing dose at time of oral dose but 200 mgm. i.v. 1 hour after.

46.0%, 38.4%; 49.0%, 54.0%; 58.4%, 54.7%; 60.0%, and 62.7%.

Effectiveness of the Flushing Dose with Larger Oral Doses

The effectiveness of the 200 mgm. flushing dose when used with larger oral doses of radioactive thiamine, was tested. Twenty-four control subjects were given either 1.0 mgm, 5.0 mgm, or 20.0 mgm.of radioactive thiamine orally together with 200 mgm. of non-radioactive thiamine intravenously. The results were compared with those obtained in the same subject, given the same test as before, but with additional 100 mgm. intravenous injections of non-radioactive thiamine at 4 hours, 9 hours, 12 hours and 24 hours after the oral does. Urine was collected for 72 hours after each test (Table 6; Figure 7). A one-way analysis of variance computed on the difference between the two tests showed that giving extra flushing doses did not significantly alter the excretion. $P[F\pm 0.704; d.f.(2,22)] = 0.99$. The giving of additional flushing doses did not significantly increase the total excretion of activity nor alter the pattern of excretion. Consequently, a 200 mgm. flushing dose was used with all oral dose levels. Approximately 45.0% of a 1.0 mgm., 34.0% of a 5.0 mgm. and 25.0% of a 20 mgm. oral dose is excreted in the urine in the first 72 hours. Attempts were made to count the radioactivity remaining in a divided, four day, stool collection by oxidising the

Table 6. The effect of giving additional intravenous flushing doses to control subjects receiving 1.0 mgm., 5.0 mgm. or 20.0 mgm. of radioactive thiamine orally

Subjects were given two tests: First* and Second**.

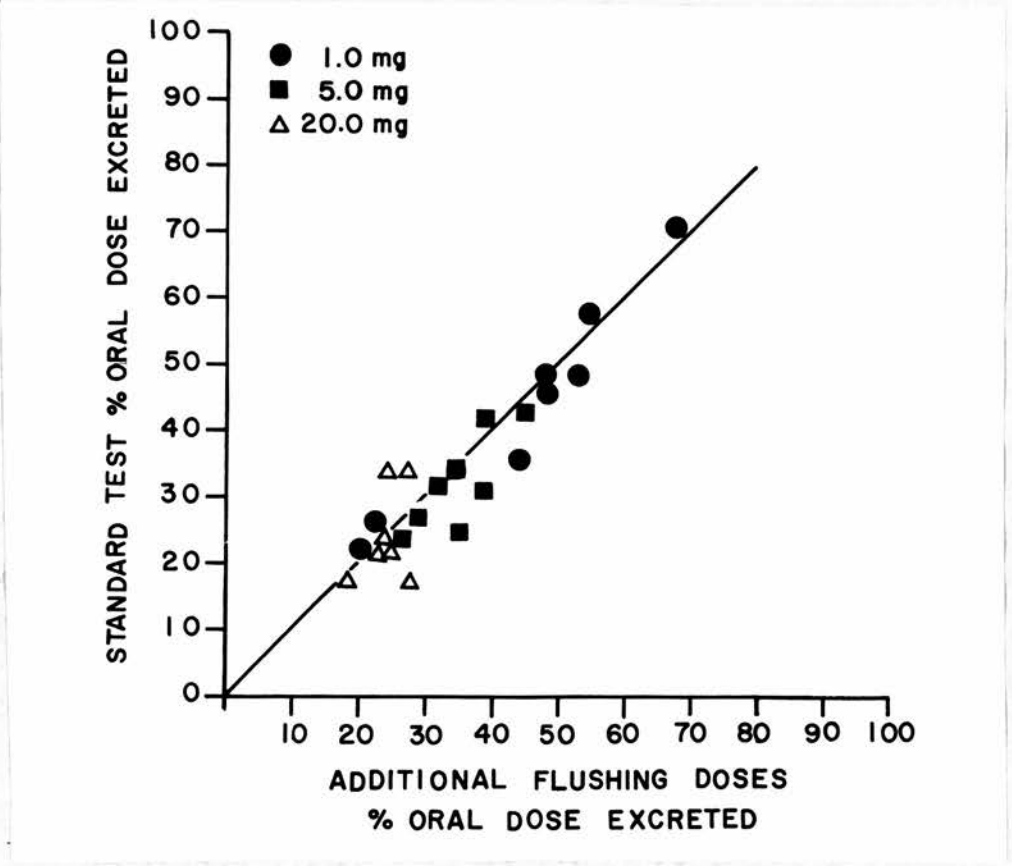
Dose 1.0 mgm.		Dose 5.0 mgm.		Dose 20.0 mgm.	
First test	Second test	First test	Second test	First test	Second test
21.7	21.2	24.9	26.7	17.5	25.8
26.0	22.9	25.1	35.6	17.8	18.4
35.5	44.9	27.9	29.2	21.7	22.5
47.2	48.7	30.8	39.5	23.4	24.5
49.0	49.0	32.9	31.7	23.5	24.2
49.0	53.0	34.2	35.1	34.2	35.2
58.4	54.7	43.1	39.3	34.3	26.1
70.9	68.5	44.8	45.4	34.8	27.0
MEAN 44.7	45.4	33.0	35.3	25.9	25.5
±SEM ±5.75	±5.65	±2.68	±2.15	±2.62	±1.68

*First test — 1.0 mgm., 5.0 mgm., or 20.0 mgm. radioactive thiamine orally and 200 mgm. non-radioactive thiamine flushing dose intravenously at the same time.

**Second test — Same as for first test with additional 100 mgm. flushing doses at 4, 9, 12 and 24 hours after oral dose.

Urine was collected for 72 hours.

FIGURE 7. The effect of giving additional intravenous flushing doses to control subjects receiving 1.0 mgm., 5.0 mgm. or 20 mgm. of radioactive thiamine (THCl) orally.


1.0 mg
5.0 mg
20.0 mg
STANDARD TEST % ORAL DOSE EXCRETED
ADDITIONAL FLUSHING DOSES
% ORAL DOSE EXCRETED
0
10
20
30
40
50
60
70
80
90
100

thiamine present with boiling nitric acid and then precipitating the sulphate as barium sulphate. However, the mass of radioactive thiamine remaining would have been extremely small and no reliable results with recovery experiments were obtained.

Figure 8 shows the cumulative excretion of radioactivity after 1.0 mgm. 35S-thiamine orally and 200 mgm. flushing dose in a control subject who previously received 300 mgm. thiamine intravenously 48 hours before. Excretion was maximal between 1-2 hours during which time 41% of the dose was excreted. By 12 hours, excretion was almost complete, 3% being added in 12-24 hours, and further small amounts thereafter (3.4% in 24-48 hour and 4.1% in the 48-96 hour periods after which excreted radioactivity was too small to be measured accurately). Various mathematical transformations of the data were attempted and it was found that when the amount excreted (in mgm. y), was plotted against log(log) time, x, an approximate straight line was obtained which could be described by the equation $y = 0.581 + 0.4247x$ (Figure 9).

A comparison of the patterns of excretion obtained at different oral dose levels is shown in Figure 10. Three subjects were given 1.0 mgm., 5.0 mgm., and 20.0 mgm. of radioactive thiamine orally and a 200 mgm. flushing dose. The maximal period of excretion occurred at approximately

FIGURE 8. The cumulative urinary excretion of radioactivity after 1.0 mgm. of 35S-thiamine hydrochloride (THCl) in a control subject; 200 mgm. of non-radioactive thiamine was given intravenously.

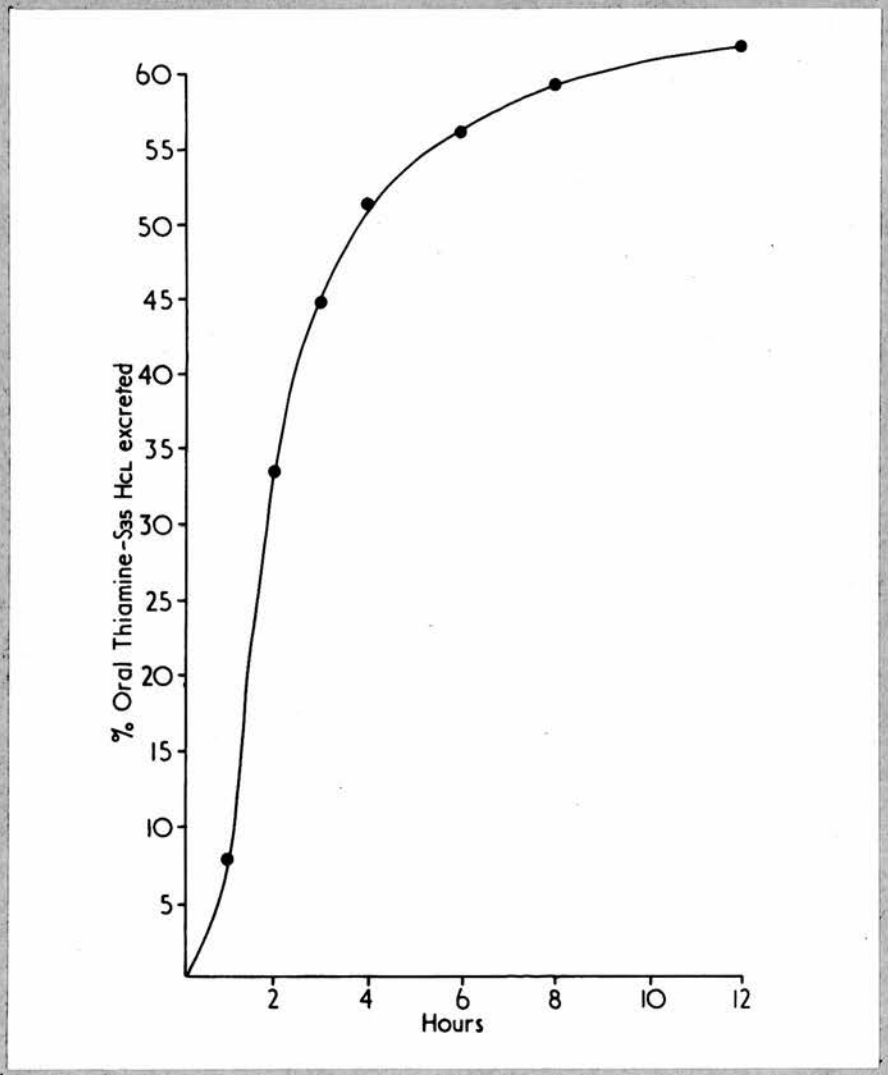

% Oral Thiamine-S35 HCL excreted
60
55
50
45
40
35
30
25
20
15
10
5
2
4
6
8
10
12
Hours

FIGURE 9. The relationship between time and the amount of radioactivity excreted following 1.0 mgm., oral dose of radioactive thiamine hydrochloride (THCl).

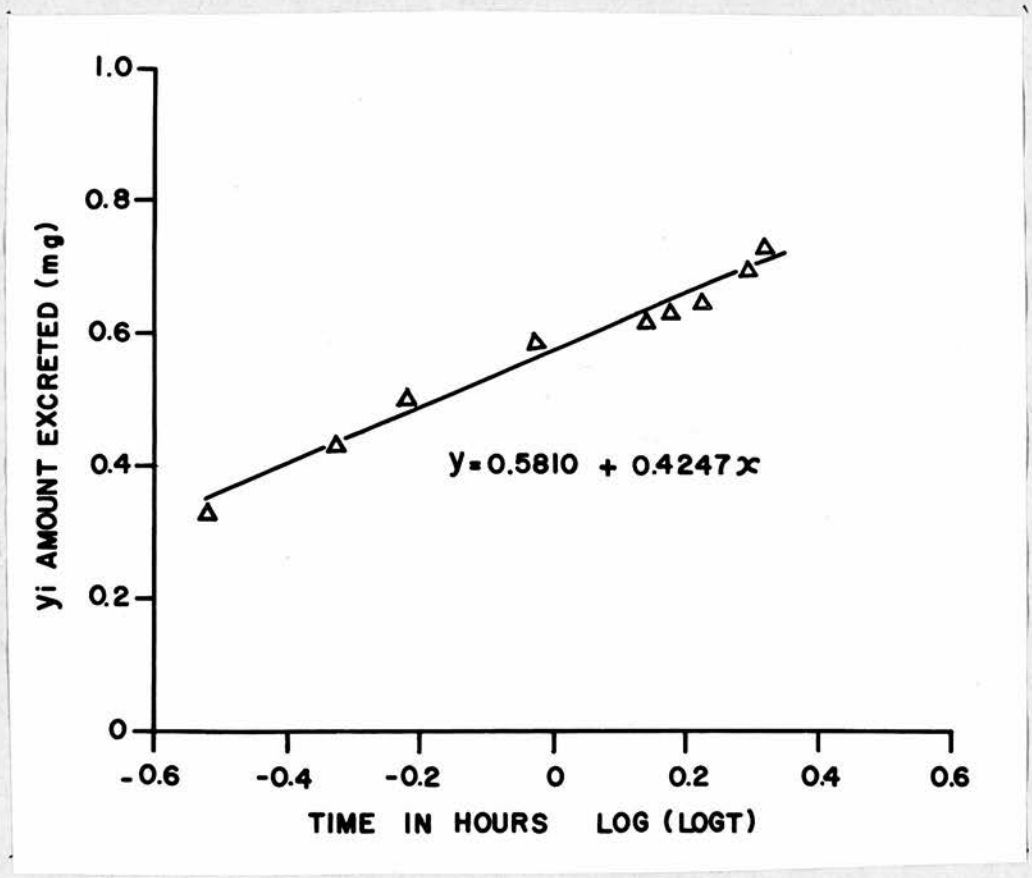
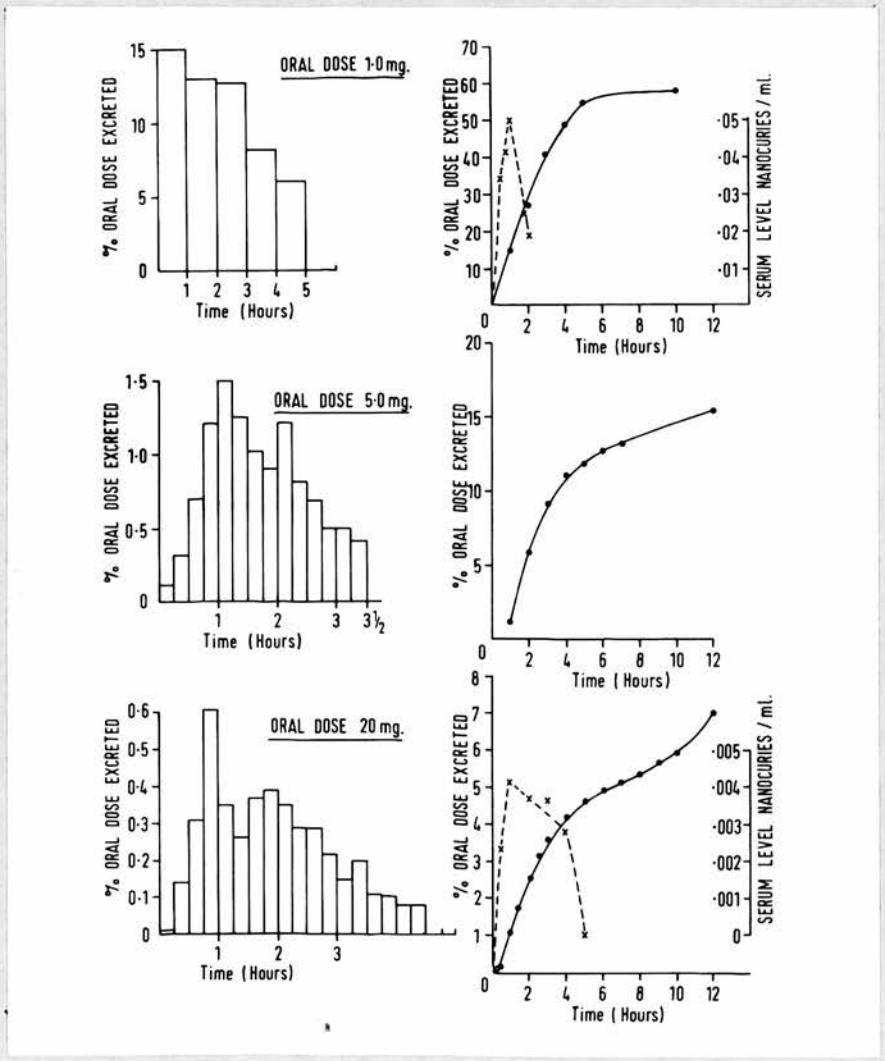

1.0
0.8
0.6
0.4
0.2
0
yi AMOUNT EXCRETED (mg)
y=0.5810 + 0.4247x
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
TIME IN HOURS LOG (LOGT)

FIGURE 10. Excretion patterns with different oral doses of radioactive thiamine hydrochloride (THCl).

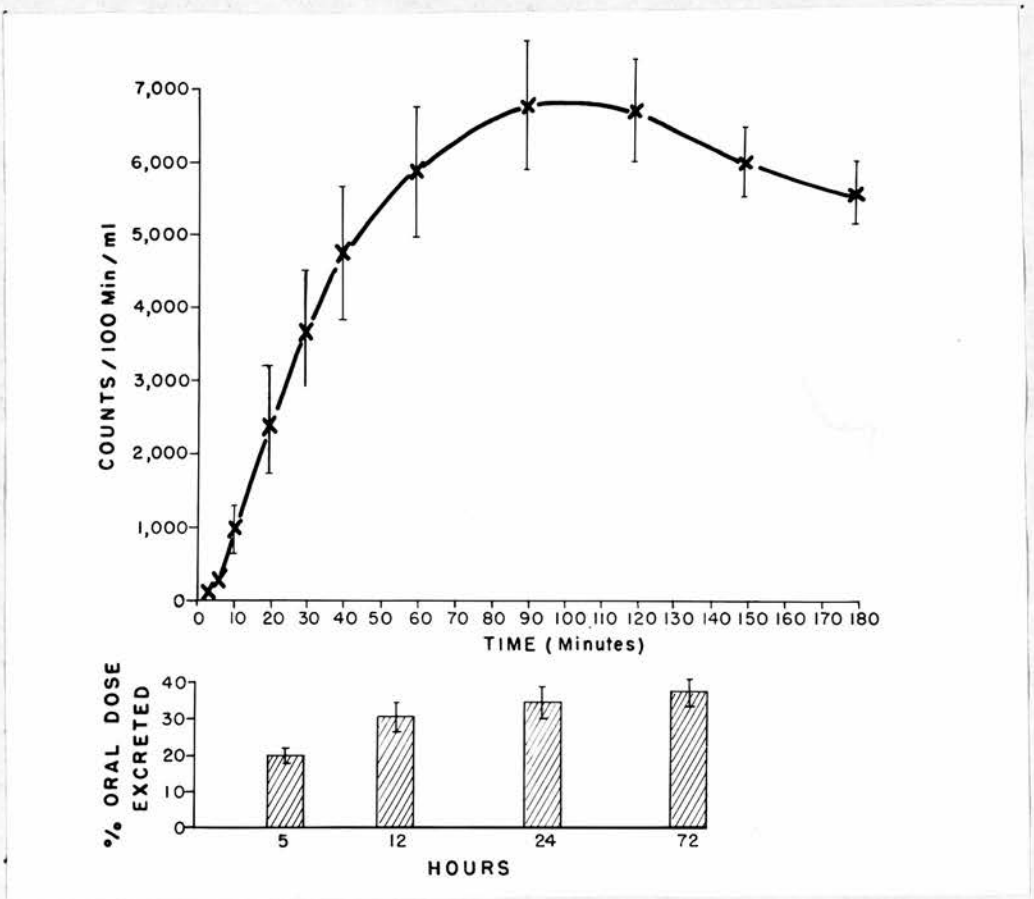

ORAL DOSE 1·0 mg.
% ORAL DOSE EXCRETED
Time (Hours)
% ORAL DOSE EXCRETED
SERUM LEVEL NANOCURIES / ml.
Time (Hours)
ORAL DOSE 5·0 mg.
% ORAL DOSE EXCRETED
Time (Hours)
% ORAL DOSE EXCRETED
Time (Hours)
ORAL DOSE 20 mg.
% ORAL DOSE EXCRETED
Time (Hours)
% ORAL DOSE EXCRETED
SERUM LEVEL NANOCURIES / ml.
Time (Hours)

the same time and the pattern of excretion is very similar in all three subjects. The mass of radioactive thiamine excreted in one hour was always extremely small when compared with the amount of the flushing dose excreted and never exceeded 0.27 mgm. The hourly outputs at the different dose levels did not increase proportionately with the size of the oral dose. During the first three hours, at 1.0 mgm. oral dose, the hourly excretions were 0.15 mgm., 0.13 mgm., 0.13 mgm.; at 5.0 mgm. oral dose they were 0.12 mgm., 0.24 mgm., 0.18 mgm., and at 20 mgm. they were 0.21 mgm., 0.27 mgm., and 0.23 mgm. At higher doses, however, thiamine continued to be excreted at a higher rate for longer.

The rate of appearance of radioactivity in the serum and the hourly urinary excretion of thiamine was tested in twelve control subjects after 5.0 mgm. 35S-thiamine (I0μc) orally and 200 mgm. flushing dose. The urinary excretions resembled the pattern shown in Figure 10. Arterial blood samples were taken at 0,3, 6,10, 20,30,60,90, 120, 150 and 180 minutes after the beginning of the test and the results are shown in Figure 11.

It will be seen that radioactivity could be detected in the serum as early as three minutes after administration of the oral dose and the maximum concentration of radioactivity in the serum was reached at approximately ninety

FIGURE 11. Arterial serum radioactivity and cumulative urinary excretion in control subjects after 5.0 mgm. 35S-thiamine hydrochloride (THCl) orally together with a 200 mgm. intravenous flushing dose.


7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
COUNTS / 100 Min / ml
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
TIME (Minutes)
40
30
20
10
0
% ORAL DOSE EXCRETED
5
12
24
72
HOURS

minutes after the oral administration. The level of radioactivity in the serum was reached at approximately ninety minutes after the oral administration. The level of radioactivity in the serum will be seen to parallel the amount excreted in the urine suggesting that urinary excretion is a sensitive indicator of changes due to the absorption of the oral dose under the conditions of the test. (Figure 10)

Comparison of rates of excretion of oral and flushing doses of Thiamine. Distribution of thiamine in extracellular fluid.

It was possible that the decline in the rate of excretion of urinary radioactivity, and the fall in serum level, two hours after an oral dose given along with a parenteral flushing dose did not reflect the absorption of radioactive thiamine from the intestine. It could have been the result of a fall in the total blood level of thiamine due to rapid excretion of the flushing dose.

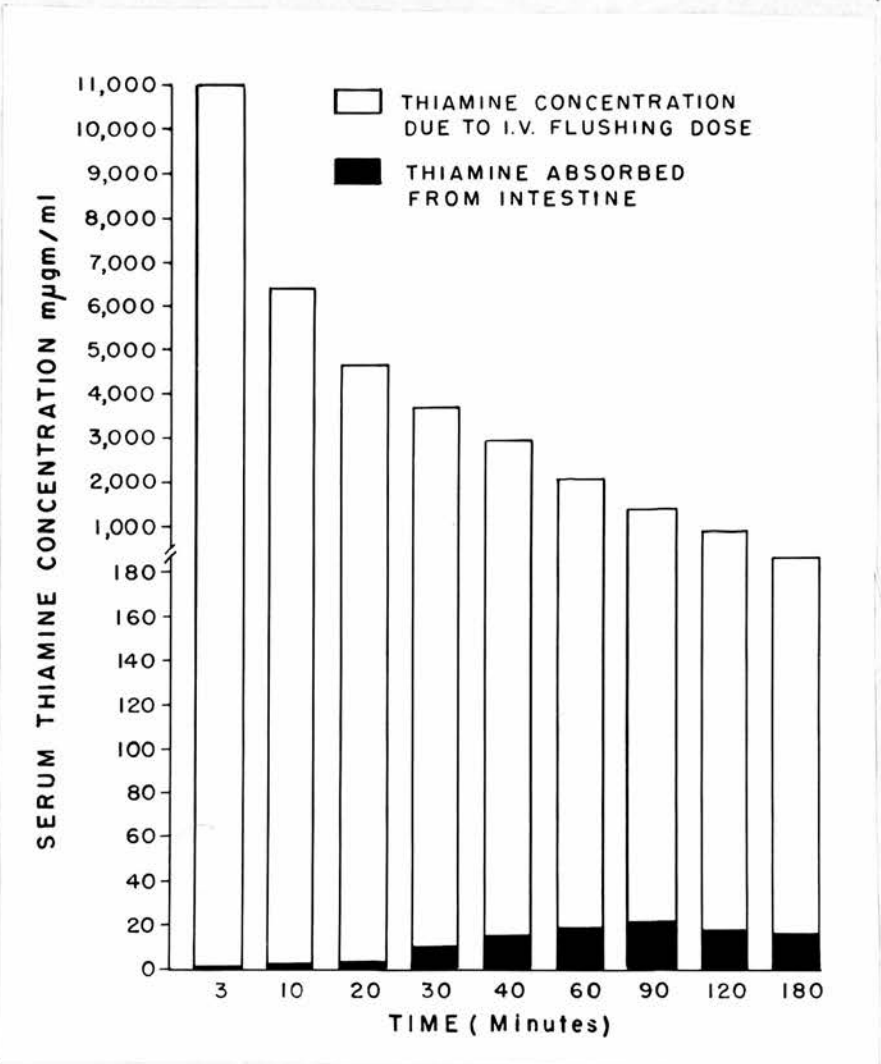
This possibility may be excluded by determining at any given time the concentration of thiamine in the serum due to the flushing dose and the concentration due to the orally administered thiamine. An estimate of these levels may be obtained by giving a subject a 5.0 mgm. oral dose of non-radioactive thiamine together with a labelled flushing dose. The test can be repeated in the same subject using a labelled oral dose and a non-radioactive

flushing dose. The arterial serum concentration of thiamine expressed as mμgm./ml. obtained using this technique are shown in Figure 12. It will be seen that the maximal level of thiamine due to the flushing dose occurs immediately following injection after which there is a steady decline in the serum level. In contrast to this, the thiamine absorbed from the orally administered dose does not reach its peak value until 90 minutes after administration. At no time does the renal mechanism for the removal of thiamine seem to be saturated. At the time of maximum absorption of the oral dose, the serum thiamine level due to the flushing dose is still one hundred times that produced by absorption from the intestine. Consequently, the patterns of radioactivity obtained in the serum and in the urine following a radioactive oral dose probably represent the true profile of absorption and the frequency with which labelled thiamine present in the oral dose undergoes metabolism or storage within the body is low.

Distribution of the administered thiamine within the body.

It is possible to determine the distribution of thiamine within the body fluids. Knowing the amount of thiamine given intravenously, and the approximate amount excreted after a given time, and the circulating volume, it is possible to predict the level that would be obtained if

FIGURE 12. Estimated concentration of thiamine in arterial blood due to absorption from the oral dose and the non-radioactive flushing dose.


THIAMINE CONCENTRATION DUE TO I.V. FLUSHING DOSE
THIAMINE ABSORBED FROM INTESTINE
SERUM THIAMINE CONCENTRATION mμgm/ml
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
180
160
140
120
100
80
60
40
20
0
3
10
20
30
40
60
90
120
180
TIME (Minutes)

thiamine were confined to the plasma volume or distributed throughout the total body water. In Table 7, the measured levels are compared to the predicted levels in the serum following a 200 mgm. 35S-thiamine hydrochloride labelled flushing dose.

It will be seen that after the initial period of mixing, the predicted results agree most closely with the observed results if it is assumed that the thiamine is distributed throughout the total body water.

Control subjects were shown to excrete approximately 35% of a 5.0 mgm. oral dose in the urine when given together with a 200 mgm. parenteral injection. However, the rise in serum concentration of radioactivity observed was comparatively small. To investigate the reason for this, rough calculations were made using mean figures obtained from the 12 control subjects studied. (Figure 11). The calculations indicate that the thiamine is partially distributed between the plasma and the extracellular fluid and that the observed values are within the range expected allowing for the rate of absorption, the rate of excretion and possible distribution of thiamine. (Tables 8 and 9)

Route of absorption

The route of thiamine hydrochloride absorption was investigated in 5 alcoholics subjects without evidence of malnutrition or liver disease, and 5 with cirrhosis, 2 of

Table 7. Measured and predicted thiamine concentrations in the serum after a 200 mgm. intravenous dose

Time (mins)	Approx % IV dose excreted.	Calculated serum conc. assum 481. total BW.* mμg/ml	Observed serum conc. mμg/ml
10	10%	3860	6360
30	30%	2920	3620
60	50%	2080	2050

*BW = body water.

Table 8. Estimated serum concentration after 5.0 mgm. of 35S-thiamine orally and 200 mgm. non-radioactive flushing dose using mean values found in the control groups

A. Level at end of one hour.

Percentage dose absorbed	≙ 5%	=	240 µgm.
Percentage of absorbed oral dose + flushing dose excreted	≙ 50%	=	120 µgm. (of oral dose)
Therefore, amount remaining in body	≙		120 µgm.
	=		120,000 mµgm.
If distributed in plasma (3,000 ml.)		=	40 mµgm./ml.
If distributed in extracellul ar fluid (12,000 ml.)		=	10 mµgm/ml.
If distributed in Total body water		=	2.5 mµgm/ml.

B. Level at end of three hours.

Percentage dose absorbed	= 10%	=	500 µgm.
Percentage excreted	= 78%	=	390 µgm.
Therefore, amount remaining in body	= 500-590		
	= 110,000 mµgm.		
If distributed in plasma volume		=	37 mµgm/ml.
If distributed in extracellular water		=	9.1 mµgm/ml.
If distributed in total body water		=	2.3 mµgm/ml.

Table 9. Summary of results - comparison of observed and predicted serum thiamine concentrations depending upon distribution within the body

Predicted concentrations mμgm/ml.

Time hr.	Average Observed serum conc. mμgm/ml	Plasma alone (31itres)	Extracellular fluid alone (121itres)	Total Body water. (481itres)
1	19.6	40	10	2.4
3	15.7	37	9.1	2.3

whom had end-to-side portacaval shunts. Absorption studies were carried out using combined umbilical and hepatic vein catheterization in the 3 malnourished patients with cirrhosis without shunts. Hepatic vein catheterization alone was used to study intestinal absorption in the others.

The Fick principle was used for estimation of hepatic blood flow, employing a constant infusion of ICG (0.3 $mg/m^2/min$), after a 10 mgm. loading dose of the dye (Leevy et al., 1962). Five mgm. of 35S-thiamine hydrochloride was given via a Rehfuss tube positioned in the duodenum prior to catheterization. Blood samples were obtained from the umbilical vein, hepatic vein, and femoral artery prior to the receipt of thiamine or ICG, at 1 minute intervals for 10 minutes, and at 10 minute intervals for 2 hours for measurement of ICG and radioactivity.

35S-thiamine was detected in hepatic venous blood 1 to 3 minutes prior to its appearance in arterial blood. Hepatic venous radioactivity remained significantly higher throughout the period of observation. In the patients with portacaval shunts, 35S-thiamine was initially detected in arterial blood and radioactivity was lower in hepatic venous than arterial blood. (Figure 13) Observations during simultaneous catheterization of umbilical and hepatic veins first revealed radioactivity in the portal vein, secondly in the hepatic vein, and finally in the femoral artery (Table 10; Figure 14).

Table.10a. Radioactivity* in hepatic vein (HV) and Femoral Artery (FA) after 5.0 mgm. 35S-thiamine hydrochloride orally and 200 mgm. of non-radioactive thiamine intravenously patients A.C. and J.P. had portal-caval shunts at the time of study

Subjects	P.Z.		P.O.		G.Y.		F.L.		D.A.		A.C.		J.P.	
	H.V.	F.A.	H.V.	F.A.	H.V.	F.A.	H.V.	F.A.	H.V.	F.A.	H.V.	F.A.	H.V.	F.A.
Time Min.														
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	1190	420	0	0	0	0	0	0	0	0	0	0
3	115	0	1340	780	0	0	0	0	0	0	0	62	0	0
4	123	0	2140	1980	212	0	0	0	180	0	0	74	0	530
5	-	42	3560	2820	500	65	0	0	460	40	0	-	132	1420
6	645	87	3140	3440	2160	825	0	0	1960	684	0	144	750	2440
7	810	289	3540	3600	3500	1480	0	0	3200	1420	0	-	1460	3160
8	970	375	3640	-	4580	2520	239	0	4300	2480	0	247	2270	4030
9	1130	740	3820	3800	6220	3200	950	83	6120	3100	0	-	2820	4730
10	1590	860	4930	4750	7400	4250	1377	476	7150	4080	0	-	3580	5530
20	4880	2610	7000	6200	1170	8550	6393	5543	9780	7120	0	409	4850	6080
30	7800	5400	8500	7550	13000	10200	10237	7496	11200	8420	0	-	7040	8810
40	10850	7450	11200	8250	12600	10300	9500	8731	12750	9900	0	-	9840	11500
60	20300	10150	9430	7450	12500	9780	9691	6452	13000	10100	0	970	14400	12300
70	24200	12500	9040	7360	11200	8200	9510	6520	12300	9700	131	1400	14300	15700
80	25200	14300	8730	7150	10300	8010	9410	6420	11300	7500	-	-	15000	15200
90	25900	15900	8250	6820	9400	7750	9379	6373	9400	7320	1000	1950	12000	11650
120	23200	13800	7100	6320	9350	7420	8488	6253	9250	7100	1500	2320	11700	9930
											3000	6500	9700	8800

A = Radioactivity = counts per 100 min. per ml.
o = No radioactivity
- = Sample not obtained

FIGURE 13. Radioactivity in hepatic vein and femoral artery after 5.0 mgm. of 35S-thiamine hydrochloride (THCl) orally and 200 mgm. non-radioactive thiamine intravenously in alcoholic subject and a patient with a portal-caval shunt.

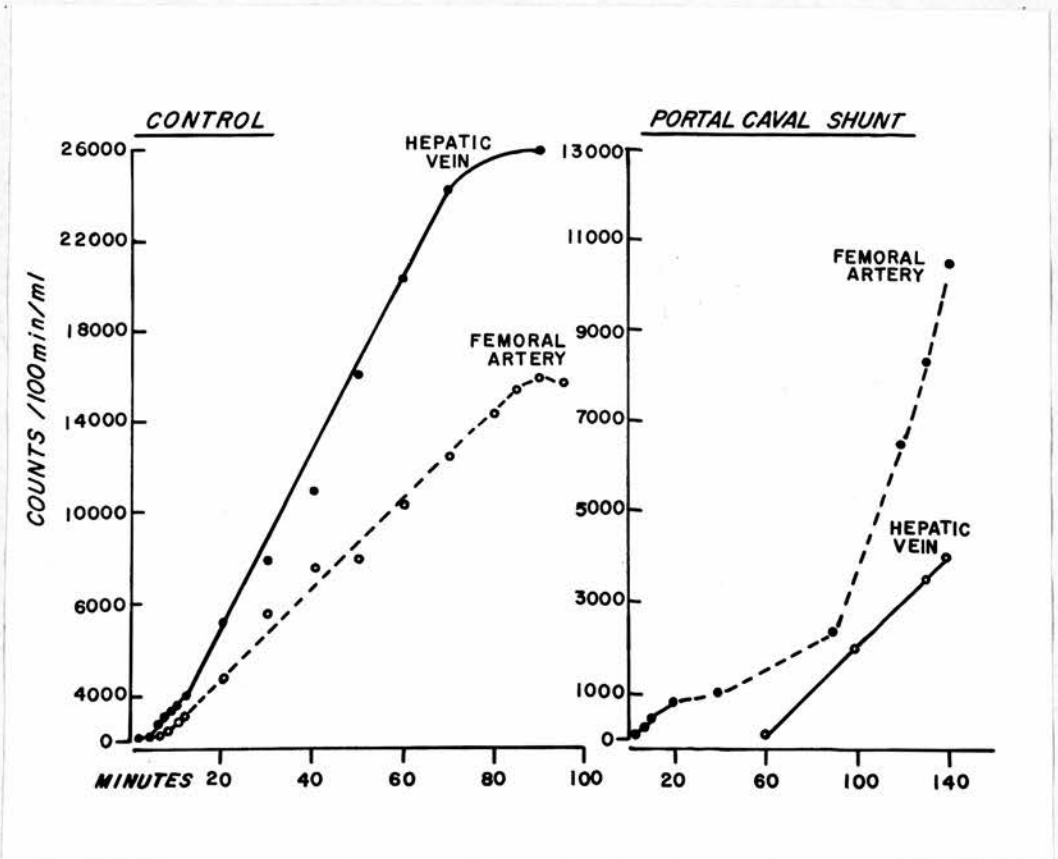

CONTROL
PORTAL CAVAL SHUNT
COUNTS /100min/ml
26000
22000
18000
14000
10000
6000
4000
0
13000
11000
9000
7000
5000
3000
1000
0
HEPATIC VEIN
FEMORAL ARTERY
FEMORAL ARTERY
HEPATIC VEIN
MINUTES 20 40 60 80 100
20 60 100 140

Table 10b. Radioactivity* in portal vein (PV), hepatic vein (HV) and femoral artery (FA) after 5.0 mgm. 35S-thiamine hydrochloride orally and 200 mgm. of non-radioactive thiamine intravenously patients A.C. and J.P. had portal-caval shunts at the time of study

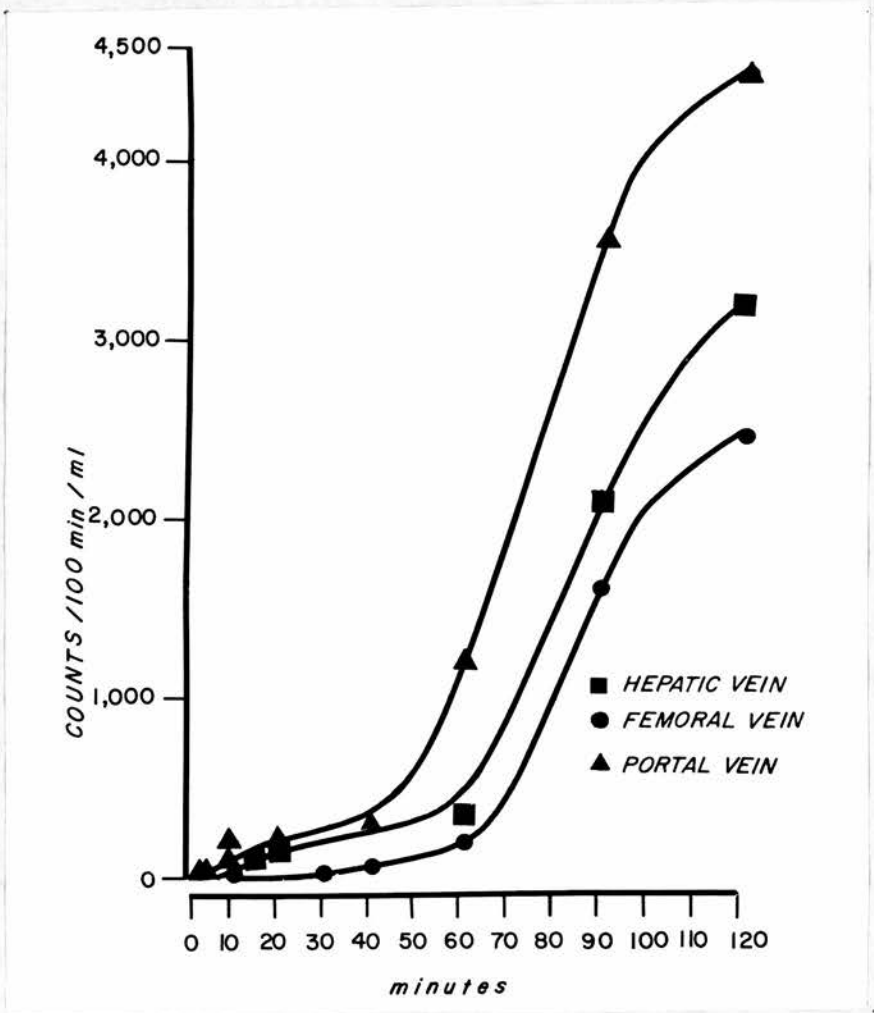
Subjects	E.Y.			F.B.			G.B.		
	P.V.	H.V.	F.A.	P.V.	H.V.	F.A.	P.V.	H.V.	F.A.
Time Min.									
0	0	0	0	0	0	0	0	0	0
1	0	0	0	180	0	0	0	0	0
2	0	0	0	440	160	98	80	34	0
3	0	0	0	1260	340	146	280	74	25
4	22	0	0	1767	470	260	480	270	90
5	34	0	0	1798	595	364	680	310	145
6	50	0	0	1832	685	410	700	480	280
7	68	0	0	1877	750	398	810	530	400
8	80	0	0	2267	830	536	900	625	480
9	98	0	0	2503	1200	571	1020	670	570
10	197	49	0	2876	1580	610	1210	850	640
20	230	160	0	3174	1820	1156	2200	1120	1020
30	264	230	39	3235	2250	1292	2650	1120	1250
40	305	292	95	2913	2470	1487	3800	1620	1400
60	1200	358	207	2379	2200	2016	4800	1870	1530
70	1920	1100	820	2180	2070	1870	5200	2200	1850
80	2570	1820	1320	2110	1980	1620	5010	2280	2010
90	3560	2140	1620	2064	1920	1512	4860	2420	2080
120	4480	3200	2490	2033	1820	1662	4500	2150	1870

* = radioactivity = counts per 100 min. per ml.

o = no radioactivity.

– = Sample not obtained.

FIGURE 14. Radioactivity in portal vein, hepatic vein and femoral artery after 5.0 mgm. of 35S-Thiamine hydrochloride (THCl) orally and 200 mgm. non-radioactive thiamine intravenously in a control subject.


4,500
4,000
3,000
2,000
1,000
0
COUNTS / 100 min / ml
0 10 20 30 40 50 60 70 80 90 100 110 120
minutes
HEPATIC VEIN
FEMORAL VEIN
PORTAL VEIN

Development of Methods to Identify Radioactivity Present in the Urine and Intestinal Contents after Oral Administration of 35S-labelled Thiamine.

It was possible that the orally administered thiamine was being broken down in the intestine and that these breakdown products were subsequently absorbed and excreted giving an erroneous measure of thiamine absorption. To investigate this possibility, methods were adapted so that the radioactivity present in the urine and in the intestinal juice, under the conditions of the test, could be identified.

Modification of phenol extraction procedure for thiamine metabolites in the urine

The method finally adopted was a modification of the phenol extraction procedure of Iacono and Johnson, 1957, and has been described in the section on General Materials and Methods. The modifications were introduced after the following experiments had been completed.

Phenol extraction. The first step in the extraction procedure was to remove inorganic salts by the phenol extraction procedure of Crammer, 1948, as these were known to cause streaking and tailing of radiometabolites on chromatograms with the various solvents employed. Experiments were carried out with different volumes of urine containing

varying concentrations of thiamine metabolites to determine the optimum ratio of water-saturated phenol-urine to be employed.

Adequate water-saturated phenol was prepared for the experiment. A 12 hour urine specimen from a patient given the standard test was thoroughly mixed and divided into as many equal volumes as required which were placed in separating funnels. Two ml. were removed to measure the total radioactivity present in each. The designated amount of water-phenol was added to each funnel, the mixture shaken vigorously, and allowed to separate overnight at 4°C. The phenol layer was then drawn off, washed several times with water to remove the last traces of salt, and a two ml. sample taken for scintillation counting. Similarly, the volume of the water layer was measured and its total activity determined. This experiment was repeated many times with urine samples from different patients and results similar to those shown in Table 11 were always obtained.

Recovery was not one hundred per cent because of difficulties in quenching experienced in counting the material and the low levels of activity in some of the specimens. However, even at a urine-water-phenol of 5:1 good extraction was obtained but equal volumes of urine and water-saturated phenol were used in the final method

Table 11. Percentage of extraction of radioactivity with varying amounts of water-saturated phenol/urine

No.	Urine Volume (ml)	Total Radioactivity in Urine (nc)*	Volume phenol added	Ratio Urine/ phenol	% of Radioactivity	
					phenol	water layer
1	300	3,550	300	1:1	94%	1%
2	300	3,660	150	2:1	94%	3.4%
3	300	3,500	100	3:1	98%	2.0%
4	58	390	58	1:1	98%	2.5%
5	58	418	46.4	5:4	93%	6.5%
6	58	350	34.8	5:3	93%	6.3%
7	58	345	23.2	5:2	92%	8.4%
8	58	375	11.6	5:1	92.5%	9.0%

*nc = nanocurries

to ensure maximal extraction.

The phenol extracts were pooled, divided into equal volumes, and shaken with a mixture of diethyl ether and distilled water in the ratio 40:1. Within minutes, the water phase containing the metabolites settled to the bottom of the funnel and were drained off. The ether-phenol mixture was washed several times with small volumes of water and the washings added to the original extract. This step in the extraction procedure produced considerable concentration of a large volume of urine. The results obtained when varying ratios of phenol extract-diethyl ether and water 40:1 were mixed with a fixed volume of urine, are shown in Table 12.

For maximal extraction, a ratio of diethyl-ether-water/phenol extract of at least 2:1 was required.

In order to eliminate still more the urinary solids and yet retain the radioactivity in solution, the urinary extract was concentrated in vacuo at 35°C to about three ml. Approximately 35 ml. of absolute ethanol were added and the volume again concentrated to four ml. The resulting precipitate did not contain any radioactivity and was discarded. This was determined by taking 0.1 ml. of urine extract and counting in the scintillation counter. Another 0.1 ml. was centrifuged at 3,000 rpm. for half an hour and the supernanant counted. Both gave identical results 47 nanocurries.

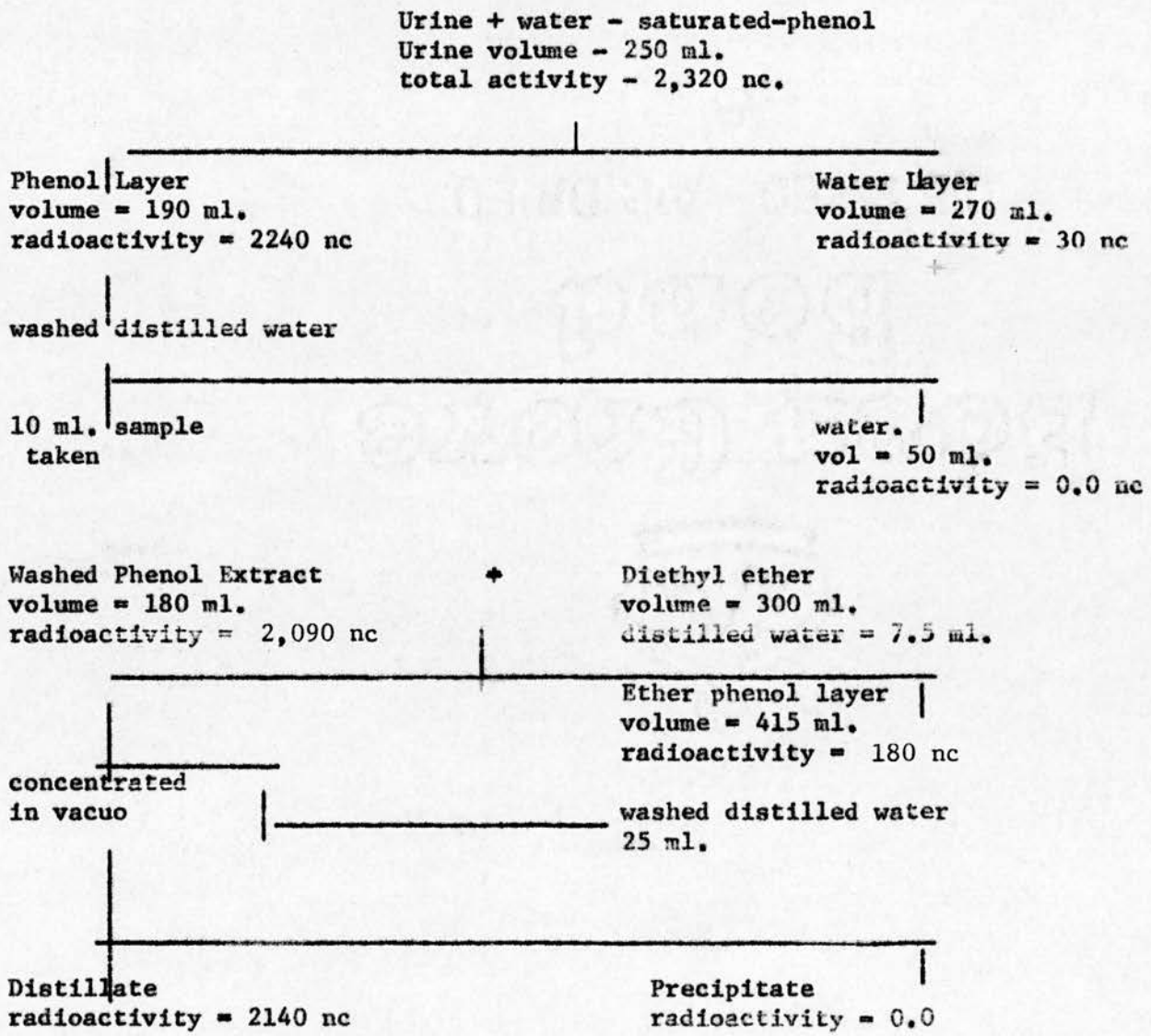
Table 12. Percentage of radioactivity extracted with varying ratios of phenolextract/diethyl ether and water 40:1

No.	Vol. Phenol (ml)	Total Radioactivity Phenol sample	Volume diethyl ether/ water added (ml)	% Radioactivity Extracted	
				water phase	phenol phase
1	100	3,430	200	91%	8.5%
2	100	3,360	100	4.5%	87.0%
3	100	3,120	25	1.4%	88.0%
4	20	341	200	97.0%	7.0%
5	20	360	150	95.0%	8.0%
6	20	304	100	97.0%	8.0%
7	20	270	50	98.0%	3.0%

The method was consistently found to remove approximately 90% of the radioactivity present in the original sample and an example of the results obtained is shown in Table 13.

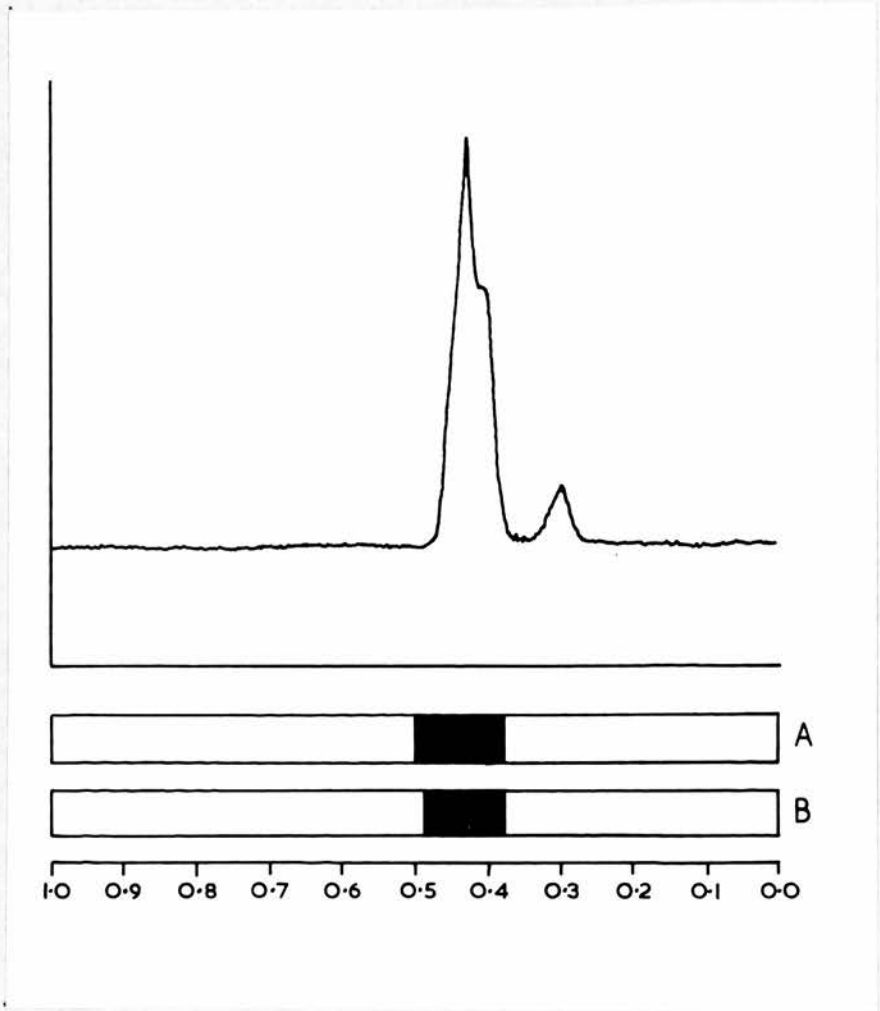
Demonstration of thiamine and its metabolites in the urine. Five patients were given 300 mgm. of non-radioactive thiamine hydrochloride intravenously 48 hours before the experiment. On the day of the experiment they received the standard test of 1.0 mgm. oral radioactive thiamine hydrochloride and 200 mgm. of non-radioactive thiamine intravenously. Urine was collected in a brown bottle surrounded by a freezing mixture of ice and solid carbon dioxide (Drikold, ICI Ltd.), during the periods of 0-1 1/2 hours, 1 1/2-3 hours, 3-12 hours. Following voiding, the urine was either analysed immediately or stored at -20°C. The radiometabolites were extracted from the urine using the modified phenol extraction procedure. Descending chromatography was performed, the papers were scanned and radioautographs prepared as described in the section of General Materials and Methods. Thiamine was identified by the thiochrome test, chromatography and bioautography as previously described. In three subjects, 90% of the radioactivity excreted during the 0-12 hour period had the same Rf. as thiamine in all three solvent systems and gave a positive thiochrome test. The eluted radioactivity could not be washed from the cells. (Figure 15). In the fourth subject, during the period 1 1/2-3 hours after the dose 90% of the radioactivity was due to thiamine

Table 13. Phenol extraction of 0-3 hour urine of a patient given 1.0 mgm. 35-Sthiamine hydrochloride and 200 mgm. flushing dose



Efficiency of Extraction - 92%

FIGURE 15. Chromatogram of phenol extract of 0-12 hour urine in a patient given 1.0 mgm. of 35S-thiamine hydrochloride (THCl) orally and 200 mgm. of non-radioactive thiamine hydrochloride intravenously.



Conditions of paper chromatography: developing solvent = n-propanol/H_2O/acetate buffer ph 5 (70: 20: 10:).

Scanning conditions: Slit 1.0 cm. Counting time 20 minutes.

Detecting Reagent: Mixture 2 parts 96% ethanol, 1 part 10% NaOH and 0.05 parts of 2.5% K_3Fe (CN) 6.

A = URINARY EXTRACT

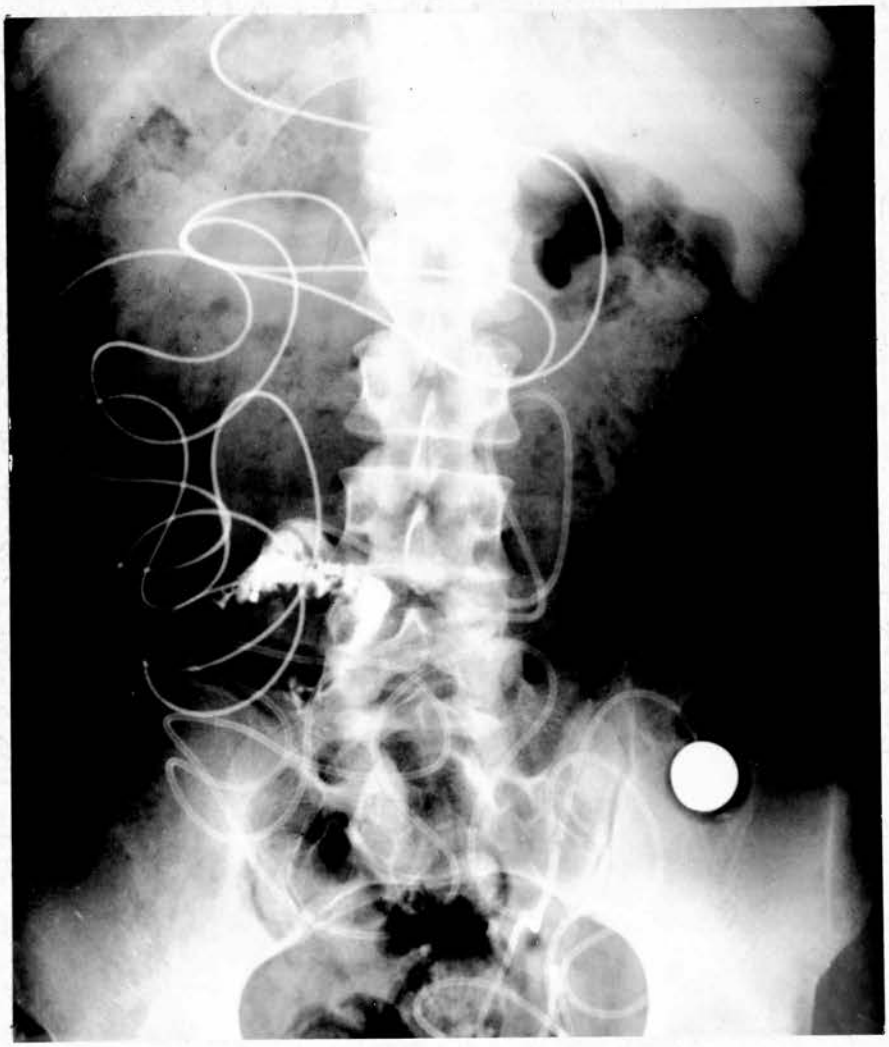
B = NON=RADIOACTIVE THIAMINE HYDROCHLORIDE

C = SCAN OF CHROMATOGRAM

but this fell to 60% in the 3-12 hour period. No attempt was made to identify the other radioactive compounds.

Extraction of radio-metabolites from small intestinal juices. Two normal subjects were intubated following an overnight fast using a 1.5 mm bore polyvinyl tube weighted at one end. The duodenal-jejunal junction is approximately 90 cm. and the ileo-caecal valve 350 cm. from the nose in adults (Blankenhorn, et al., 1955). Sampling in one subject was from points 105 cm. and 195 cm. from the nose on two successive days, i.e. from the jejunum and from the proximal ileum; in the other, sampling was only from the proximal ileum. The fasting subjects were fed a test meal consisting of "Humanised Trufood" (Trufood Ltd., The Creamaries, Wrenbury, Cheshire) and 5μc of 35S-thiamine with sufficient non-radioactive thiamine to give a final thiamine content of 1.0 mgm. The small intestinal juice was obtained by continuous sampling into a flask surrounded by a freezing mixture. The volume of intestinal juice decreased considerably after the third hour. Consequently, the observations relate to the 0-3 hour period. the pH of the intestinal juice was measured with a glass electrode and never rose above pH 6.1, i.e. it was always within the range at which thiamine is stable. This agrees with the findings of Watson and Paton, 1965. At the end of the experiment, gastrografin was introduced into the tube to check the position of the sampling point. (Figure 16)

FIGURE 16. X-ray photograph showing the tube used for sampling small intestinal juice in position. The mercury bag is seen and the more proximal sampling port identified with gastrografin.



Twenty ml. of small intestinal juice were dialysed against three 100 ml. portions of distilled water left for 24 hours at 4°C. The dialysates were freeze-dried and then dissolved in 30 ml. absolute ethanol. The solution was concentrated in vacuo at 35°C. to about 4 ml. and a sample chromatographed. Recovery experiments gave 100% recovery up to twice the concentration found in the intestine. No radioactivity was detected within the sac after dialysis and it was found that over 90% of the radioactivity was identical, chromatographically, with thiamine.

Invitro incubation of thiamine with gastric juice, bile, and small intestinal juice

Gastric juice was collected from five control subjects undergoing maximum histamine test meals and bile was obtained from four patients with T-tube drainage following common bile duct exploration and gall-bladder surgery. Samples drained into recepticles surrounded by a mixture of ice and solid carbon dioxide (Drikold, ICI, Ltd.). Rat bile was also obtained from 200 gm. Sprague Dawley rats by cannulation of the bile duct. Jejunal samples were obtained following a meal of Humanised Trufood or low protein diet from three control subjects intubated on six different occasions. The method of intubation and collection of samples was as previously

described. The level of sampling from the nose and the time after the meal was taken is summarised in Table 14.

10 ml. samples of gastric juice, bile or small intestinal juice were incubated at 37°C. with 0.12 mgm. (5.0μc.) or 1.7 μgm.(70 nc.) of radioactive thiamine hydrochloride for 4 hours, 8 hours, 12 hours and 16 hours. The samples were then chromatographed, subjected to the thiochrome test and scanned with the BTL Radioactive Chromatogram Scanner. In all cases, the radioactivity was present in only one spot which gave a positive thiochrome reaction and had the same Rf as thiamine in all three solvent systems. Consequently, under the conditions of the test, no breakdown of thiamine was detected up to 16 hours incubation at 37°C.

The influence of renal disease on the excretion of absorbed thiamine.

Eight patients with severe renal disease were investigated to determine the influence of renal impairment on the standard test. The subjects were given either 1.0 mgm. or 20.0 mgm. of radioactive thiamine orally and 200 mgm. of non-radioactive thiamine intravenously. Absorption was studied by the appearance of radioactivity in the venous blood and the urinary excretion of radioactivity in divided 72 hour urine samples. Some patients were given 200 mgm. of thiamine intravenously alone containing 10 μc 35S-thiamine

Table 14. Collection of samples of intestinal juice from different regions of the intestine in three control subjects

Subject	Test	Distance from Nose (cm)		Period of Sampling	Total Volume (ml.)	pH	Meal Taken
		Start	Finish				
I.	A.	79.0	97.0	First hour	221	6.05	HT.*
				Second hour	223	5.18	
				Third hour	91	5.17	
	B.	144	157	First hour	140	6.13	HT.
				Second hour	172	6.04	
				Third hour	90	5.55	
2.	A.	60	60	First hour	248	4.42	Low Prot.**
				Second hour	202	4.20	Low Prot.
				Third hour	18	4.39	Low Prot.
	B.	130	130	First hour	50	6.28	HT.
3.	A.	120	160	First hour	55	5.86	HT.
				Second hour	71	5.98	
				Third hour	63	5.90	
	B.	189	250	First hour	45	6.29	HT.
				Second hour	135	6.38	
				Third hour	29	6.32	

*HT = Humanised Truefood.

**Prot. = Protein.

to investigate the rate of clearance of the flushing dose from the blood. The results are shown in Table 15. The urinary excretion of radioactivity was markedly reduced in some patients even after 72 hours of collection; the lowest levels being associated with the most severe degrees of renal impairment. The patients could not be used as their own controls since normal renal function never returned. However, the patients with creatinine clearances greater than 35/40 ml./min. excreted normal amounts of radioactivity. Even in these patients, some delay in the rate of excretion can be detected (Figure 17) which increases as the renal function deteriorates. Venous serum levels of radioactivity in these patients showed peak values between 1-2 hours comparable to those found control subjects but the serum levels remained elevated for a longer period. This was associated with a delay in excretion of the flushing dose (Table 16; Figure 18). Since the peak concentration in the serum occurs approximately at the same time as in control subjects, delayed excretion secondary to reduced renal function possibly explains the reduced urinary excretion of radioactivity rather than a decrease in intestinal absorption.

Table 15. Percentage of radioactivity excreted after a 1.0 mgm. or 20.0 mgm. oral dose together with 200 mgm. flushing dose in patients with renal disease

No.	Age	Sex	Diagnosis	Drugs	Blood Urea mgm.%	Creatinine Clearance	%Radioactivity Excreted after 1.0 mgm.	20 mgm.
1.	46	M	Chronic Pyelonephritis	-	200	4ml./min. 3ml./min.	5.4%	-
2.	69	M	Hypertensive Renal Dis.	Guaneth-idine	150	8ml./min. 6ml./min.	27.6%	-
3.	51	M	Glomerulo-nephritis 'minimal leision'	Prednisone 60 mgm. daily	82	29ml./min. 27ml./min.	40.0%	36.6%
4.	64	M	Nephrotic Syndrome Gout	-	70	30ml./min. 31ml./min.	24.3%	16.7%
5.	63	M	Diabetes	Insulin	70	32ml./min. 33ml./min.	55.4%	18.3%
6.	40	M	Diabetes	Insulin	80	43ml./min. 40ml./min.	43.1%	32.3%
7.	54	F	Membraneous Glomerular nephritis	Lasix Prednisone 5 mgm. bid.	89	59ml./min.	-	27.9%

FIGURE 17. Cumulative urinary excretion of radioactivity after 1.0 mgm. of 35S-thiamine hydrochloride (THCl) orally and 200 mgm. non-radioactive thiamine intravenously in three patients with severe renal disease.

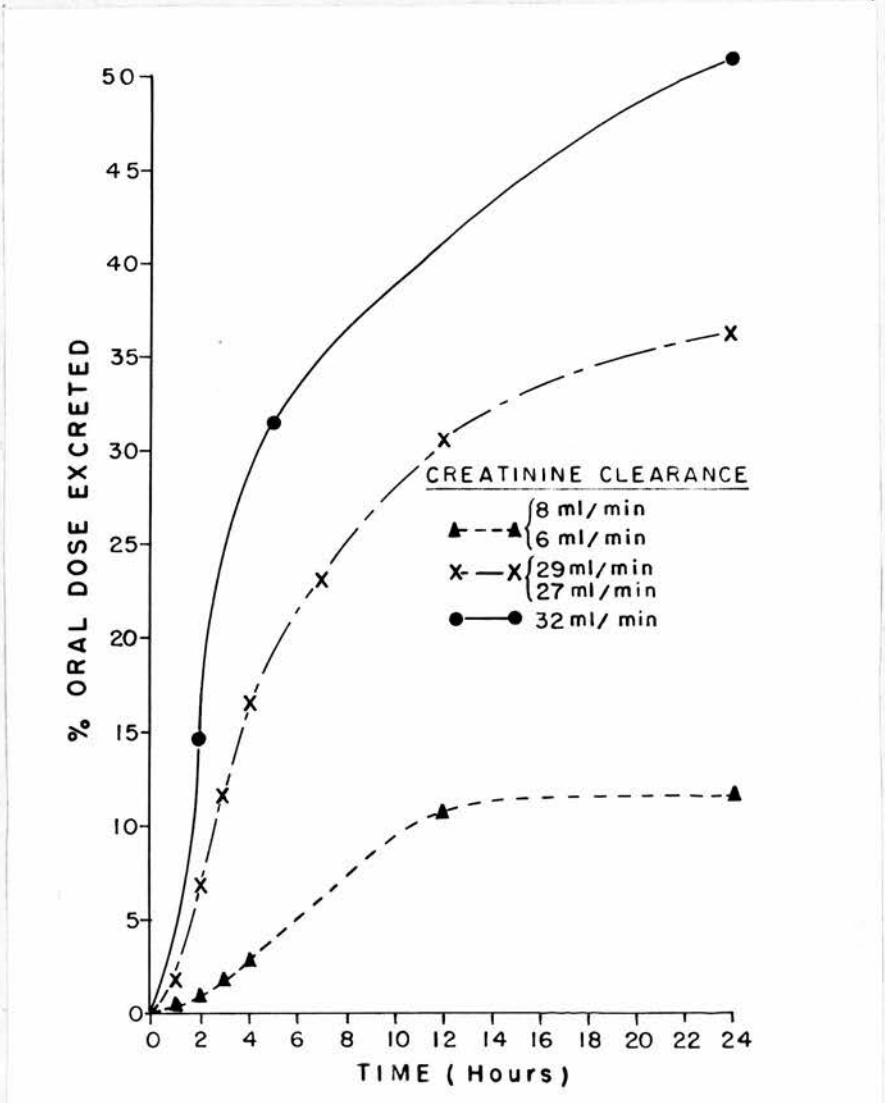

% ORAL DOSE EXCRETED
50
45
40
35
30
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16 18 20 22 24
TIME (Hours)
CREATININE CLEARANCE
8 ml/ min
6 ml/ min
29 ml/ min
27 ml/ min
32 ml/ min

Table 16. Urinary excretion of radioactivity after 200 mgm. of 35S-thiamine intravenously in a control subject and two patients with renal impairment

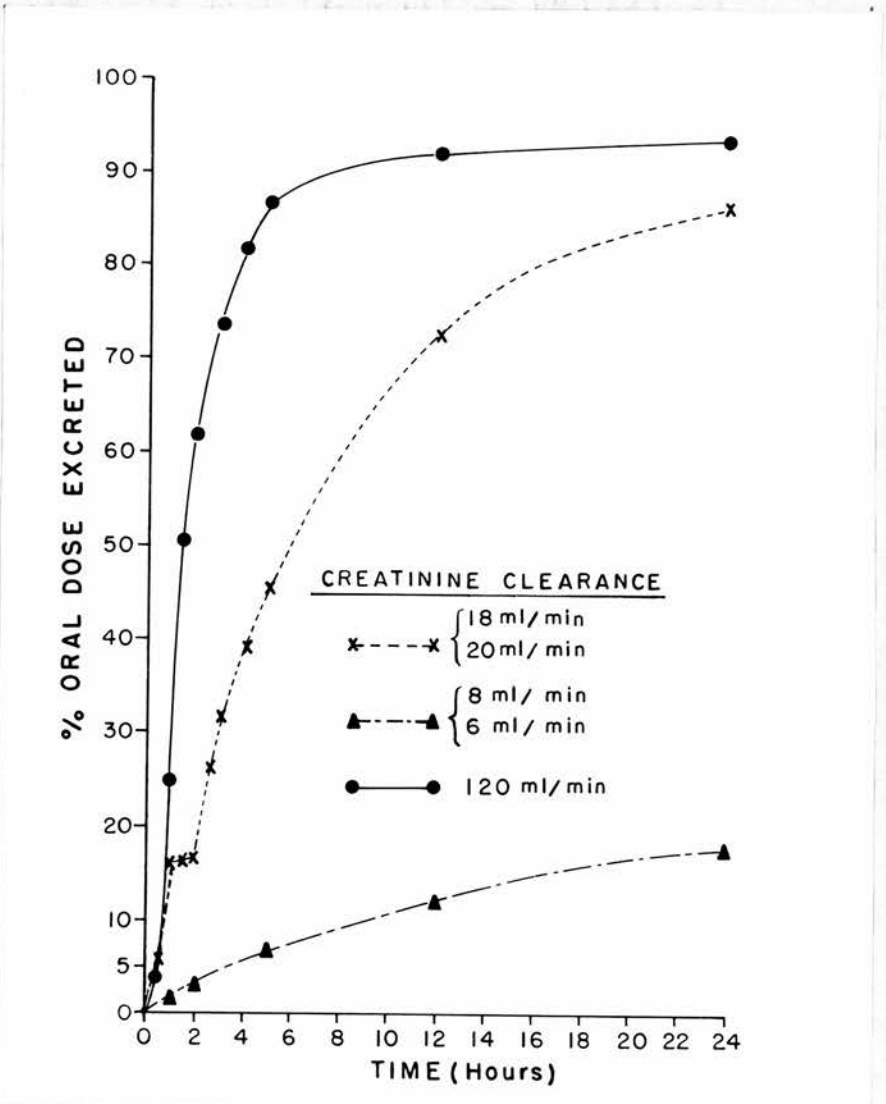
Time (hr.)	%200 mgm. IV dose excreted		Time (hr.)	%200 mgm. IV dose excreted
	Control*	Chronic** Pyelonephritis		Hypertensive*** Renal Dis.
0-.5	3.8	5.6	0-1	1.6
.5-1	21.1	10.4	1-2	1.3
1-1.5	25.6	0.1	2-5	4.0
1.5-2	11.4	0.2	5-12	4.8
2-2.5	6.1	9.7	12-24	6.0
2.5-3	4.9	5.7		17.8
3-4	8.1	8.5	24-48	28.5
4-5	4.7	6.5		
5-12	5.3	26.6		
12-24	2.3	13.8		
	93.3	87.3		46.3

*Creatinine Clearance - 120ml./min.

**Creatinine Clearance - 18ml./min.
20ml./min.

***Creatinine Clearance - 8ml./min.
6ml./min.

FIGURE 18. Cumulative urinary excretion of radioactivity after 200 mgm. of 35S-thiamine hydrochloride (THCl) intravenously in a control subject and in two patients with renal impairment.


% ORAL DOSE EXCRETED
100
90
80
70
60
50
40
30
20
10
5
0
0 2 4 6 8 10 12 14 16 18 20 22 24
TIME (Hours)
CREATININE CLEARANCE
18 ml/min
20 ml/min
8 ml/min
6 ml/min
120 ml/min

DISCUSSION

These investigations indicate that radioactive thiamine can be used to study the absorption of this vitamin in man. Analysis of small intestinal juice and extraction of the radioactivity present in the urine, shows that during the period when maximal absorption is taking place, little breakdown of thiamine occurs in the small intestine and that most of the radioactivity obtained in the urine is thiamine which was unchanged prior to absorption.

When a relatively large injection of non-radioactive thiamine is given intravenously, the percentage excretion of radioactivity rises from about 6.0% to over 50.0%. Since the non-radioactive parenteral thiamine promotes the urinary excretion of the labelled vitamin, it would seem that the radioactive thiamine behaves similarly to the non-radioactive thiamine during its passage through the body. Increasing the flushing dose to 300 mgm. thiamine does not produce any further increase in the percentage urinary excretion of radioactive thiamine (Table 3; $p<0.5$) and it would therefore seem unlikely that the flushing dose is altering the normal physiological activity of the intestine.

Although one control patient excreted 90.7% of the

oral dose within the first 24 hours, the mean excretion for the group was only 54.1% (SEM ± 5.66) and prolonging the urine collection for nine days in one subject added only a further 7.5%. The total thiamine content of the human body has been estimated to be 30 mgm. (Takeda, 1947).

Evidence suggests that in man a subject with normal circulating thiamine levels can develop symptoms due to thiamine deficiency after three weeks on a thiamine free diet. Leevy et al, 1965b, monitored the decline in serum thiamine and urinary thiamine which occurred in an obese 29 year old woman during 188 days of starvation. Lassitude, hypotension and hyperemesis developed in the third week which was only relieved by intravenous thiamine. The minimum daily requirement of thiamine in the adult human has been estimated at between 0.23 mgm/1000cal and 0.66mgm/1000cal (Williams et al, 1942; Oldham et al, 1944; Daum et al, 1949; Dick et al, 1958 and Ziporin et al, 1965).

The findings of Leevy et al, 1965b, are consistent with the estimate of Takeda, 1947, suggesting that the body stores are approximately 30 mgm.

If the body stores are small, it should be possible to saturate them and provide the body's requirements by giving an adequate intravenous dose of thiamine and so prevent retention of the orally administered radioactive thiamine.

Approximately 0.05 mgm. of radioactive thiamine is contained in the oral dose. An attempt was made to reduce any exchange with the body stores by previous loading of the patient and by the provision of additional flushing doses each of 200 mgm. of non-radioactive thiamine to dilute the absorbed radioactive thiamine. However, giving additional flushing doses, or variation in time or route of administration of the flushing dose, did not significantly increase the total 24 hour excretion of activity nor alter the pattern of excretion, following 1.0 mgm. to 20.0 mgm. of oral thiamine. Previous studies have shown that subjects on an ordinary diet with thiamine content of about 1.5 mgm. usually excrete between 0.4 and 1.0 mgm. of thiamine daily in the stool (Schultz, et al, 1938; Youmans et al, 1940; Friedmann, et al, 1948; Alexander and Landwehr, 1946; Denko et al, 1946; Yano, 1958). Consequently, it would seem that most of the 1.0 mgm. oral dose absorbed is excreted during the first 24 hours, under the conditions of the test. Further support of this conclusion is provided by the observation that more than 90% of an administered 200 mgm. intravenous flushing dose was recovered in the urine within the first 24 hours. There is little evidence of re-excretion of thiamine into the intestine as observed in rats by Gassmann and Ketz, 1961, under the conditions of the test. The total excretion of thiamine in the bile

as measured by Ochromonas danica in bile obtained from a patient with T-tube drainage, was only 110 µgm. total in 24 hours after an intravenous dose and 70.4 µgm. after an oral dose of 15 mgm. of thiamine hydrochloride. (Baker et al, 1968). Haugen (1961), showed that excessive amounts of thiamine are rapidly excreted by the kidney and in the present investigation there was no evidence that at any time the kidneys were unable to excrete the load presented to them.

In control subjects, the major period of absorption was in the first two hours after the 1.0 mgm. oral dose (Figure 8). This was also true at higher oral dose levels although the period of absorption was prolonged (Figure 10). At all dose levels, the rate of appearance of radioactivity in the serum anticipated, by a very short interval of time, the pattern of excretion seen in the urine. The 200 mgm. intravenous flushing dose appeared to distribute itself throughout the total body water 30 minutes after administration. The low serum levels obtained from orally administered thiamine were explicable if the rate of absorption and the rate of excretion of the total body thiamine were taken into consideration. The distribution of the orally administered thiamine seemed to be modified by the presence of the flushing dose and appeared to be mainly limited to the extracellular fluid. A straight line

relationship was found to exist between the amount of thiamine excreted after 1.0 mgm. oral dose together with 200 mgm. flushing dose (y) and log(log) time (x) which could be described by the equation y= 0.5810 + 0.4247x.

Radioactivity has been detected in the serum within the first three minutes in control subjects following orally administered 35S-thiamine and small amounts have been detected in the urine in the first five minutes in some subjects. This suggests that thiamine is absorbed high in the intestinal tract. Studies using the technique of combined umbilical and hepatic vein catheterization, demonstrated consistently that following an oral dose the serum radioactivity first rises in the portal vein, then in the hepatic vein and finally in the femoral artery. At all times, the concentration of radioactivity is greater in the portal vein than in the hepatic vein and lowest in the femoral artery. These observations indicate that thiamine is absorbed via the portal system rather than via the lymphatics.

The amount of thiamine appearing in hepatic venous blood depends upon its extraction ratio. Provision of a large saturating dose of thiamine prevented significant retention in both deficient and repleted subjects. Several factors contribute to the observed portal-hepatic vein thiamine gradient including tissue equilibration, arterial

inflow, and extracellular distribution of the vitamin. As previously stated, preliminary data indicate less than 1% of a 15 mgm. dose of radioactive thiamine is recovered from the bile over a 24 hour period. Absorption via the portal system was previously suggested by Bean et al, 1951, who sampled from an anastomotic abdominal vein and a peripheral vein in two cirrhotic patients. An evaluation of the data was presented in the introduction. The work of Middleton and Grice, 1964, in the rat indicates that the site of maximum absorption is in the duodenum and upper small intestine and this agrees with the findings of Polin et al, 1964, in the chick.

The results of the test must be carefully interpreted in patients with severe renal disease. When the creatinine clearance is below 35 ml./min., both the rate of excretion and the total amount excreted may be reduced. This is not thought to be secondary to malabsorption of thiamine since the peak radioactive value occurs in the serum between 1-2 hours. In patients with creatinine clearances greater than 35 ml./min. there may still be some delay in the rate of thiamine excretion, but this becomes less marked as the renal disease becomes less severe. Because of the small number of subjects, it was not possible to relate the reduction in thiamine excretion to the region of the kidney involved in the disease process.

Comment

Many of the quantitative tests used to measure vitamin B1 absorption in man have been unsatisfactory either because they were unable to measure thiamine and its metabolites accurately or because unknown amounts of absorbed thiamine were being incorporated into the body stores or utilized in body metabolism erroneously suggesting reduced absorption. The limitations of methodology made the interpretation of data extremely hazardous and no reliable test to measure thiamine absorption has been developed which can be used routinely. Evidence presented here indicates that the use of 35S-thiamine hydrochloride with an appropriate flushing dose, provides a simple and reliable method for measuring thiamine absorption in man.

CHAPTER II

THE INFLUENCE OF GASTRO-INTESTINAL DISEASE ON THIAMINE ABSORPTION

Introduction

Disease of the small intestine may cause impaired absorption of certain water-soluble vitamins. Patients with primary malabsorptive disease (idiopathic steatorrhoea), for example, frequently fail to absorb folic acid normally (Girdwood, 1953; Girdwood & Delamore, 1961; Chanarin, et al., 1958; Cox, et al., 1958). Although clinical evidence of deficiency of other water soluble vitamins is rarely present, investigations using the tryptophan loading test have suggested subclinical deficiency of pyridoxine in patients with tropical sprue and primary malabsorptive disease (Kowlessar et al., 1961; Sigler et al., 1962). Baker & Sobotka (1962) have shown that patients with primary malabsorptive disease may have subnormal levels of serum pyridoxine and Brain & Booth (1964), using tritium-labelled pyridoxine, have demonstrated impaired absorption of pyridoxine in some of these patients.

Girdwood (1956), using microbiological methods, studied the absorption of water soluble vitamins in

patients with primary malabsorptive disease, but found no evidence of malabsorption of thiamine, riboflavin or pyridoxine in 10 patients with impaired absorption of folic acid. Except for this investigation, little has been published on the absorption of thiamine in patients with intestinal malabsorption.

Methods and subjects

Patients with primary malabsorptive disease included 10 cases who had not been treated with a gluten free diet. The diagnosis was established by jejunal biopsy and the results of the following absorption tests - folic acid absorption (Girdwood, 1953; Girdwood & Delamore, 1961), vitamin B_{12} absorption (Schilling, 1953), xylose absorption (Fourman, 1948) and faecal fat excretion (Kramer et al., 1949). A sternal marrow was performed and serum folate, formiminogluatmic acid (FIGLU), and vitamin B_{12} levels were measured. All patients had a barium meal and follow-through examinations. There were also 13 patients who had been treated with a gluten free diet for periods varying from 3 months to 5 years.

The other patients included 10 with gastroenterostomies 8 with partial gastrectomies and 3 patients with pernicious anaemia who had confirmed acid-fast achlorhydria in response to a maximum histamine test meal and were untreated at the time of investigation. Three patients had

Crohn's disease and one who had had a sub-total proximal gastrectomy with an end-to-side oesophago-gastric anastamosis for an anaplastic carcinoma of the stomach 17 years previously, but there was no postmortem evidence of a recurrence when she died from a myocardial infraction some months after completion of the studies. Two of the patients with Crohn's disease had had a resection of the terminal ileum and a right hemicolectomy.

Standard Test. Most of the patients studied in this section were given the standard test of i.e. 1.0 mgm. oral dose of 35S-thiamine hydrochloride together with 200 mgm. of non-radioactive thiamine intravenously. Divided collections of 24 hour urines were made.

RESULTS

Primary Malabsorptive Disease. The excretion of eight patients with primary malabsorptive disease given 300 mgm. intravenously 48 hours before the standard test was 26.7%(SEM ± 5.11) which was significantly less than the control group ($t = 3.504$; $p<0.01$). When the 300 mgm. "loading" dose was omitted there was no decrease in the recovery of urinary radioactivity 28.1%(SEM ± 4.75) Tables 17; 18. The difference between these patients and the controls was not due to a decreased ability to excrete thiamine as a patient was able to excrete an

Table 17. Thiamine absorption in patients with primary malabsorptive disease

Standard test repeated in the same subject after giving 300 mgm. thiamine intravenously 48 hours before the second test.

Subjects	No. of subjects	Before loading	After loading
Untreated	8	28.1 ± 4.75	26.7 ± 5.11
Treated	13	36.9 ± 4.33	40.8 ± 3.84

The results are expressed as the mean ± SEM (n)

Loading was achieved as before (See Table 2)

Table 18. 35S-Thiamine HCl absorption in untreated primary malabsorptive disease before and after saturation

Age	Sex	Hb. g/100ml	P.C.V. %	Marrow	Serum folate ng/ml	FIGLU	Folic acid absorption*	Serum Vit.B_{12} pg/ml	Vit. B_{12} absorption % oral dose	Faecal fat g/day	35S-THIAMINE HCl ABSORPTION Urinary Excretion of radioactivity (%) Before Saturation	After Saturation	Intestinal Mucosa
62	M	13.6	43	-	12.2	-ve	-	368	Malab. 3.9%	69.5	9.0	7.2	Subtotal villous atrophy
68	F	9.5	32	Meg.	4.0		Malab.	175	Malab. 2.34%	3.6	11.0	9.4	Subtotal villous atrophy
53	M	12.5	42	Meg.	6.8	-ve	-	158	Malab. 1.9%	12.6	30.0	21.0	Subtotal villous atrophy
34	F	12.5	32	Meg.	3.4	+ve	Malab.	299	Normal 12.2%	2.6	26.5	25.2	Subtotal villous atrophy
62	F	12.4	41	-	4.2		Malab.	196	Malab. 3.0%	7.7	29.0	28.6	Subtotal villous atrophy
29	F	8.5	32	Meg.	3.9	+++	Malab.	183	Malab. 6.5%	8.5	35.5	32.9	Subtotal villous atrophy
38	F	11.2	40	Meg.	2.7	+ve	Malab.	248	Malab.	47.0	32.8	38.5	Abnormal
18	F	8.4	30	-	3.3	+ve	Malab.	235	-	3.6	51.0	50.5	Failed
										MEAN	28.1	26.7	
										SEM±	±4.75	±5.11	
Normal values		13.6-16.0			4.5-8.0			<196	<12.1%	>5.0			

Malab. = malabsorption.

*Measurement folic acid absorption by comparing urinary output of folic acid after injected (a) and oral (b) doses. Malabsorption = output less than 1.5 mgm. after oral dose, together with excretion index (B/A x 100) of less than 75%.

intravenous dose normally.

Rate of Urinary Excretion

The cumulative excretion of radioactivity in a "loaded" untreated patient showed delay in excretion and marked reduction in the total excreted 9.4% although maximal excretion occurred within the first 6 hours. (Figure 19) When this test was repeated in the same subject, 11.0% of the oral dose was recovered and the pattern of excretion was identical.

Additional Flushing Doses given to three malabsorptive patients during the 0-24 hour period and to two others during the 24-48 hour period did not alter the pattern of excretion or significantly increase the total radioactivity excreted; the details of one patient are shown in Table 19.

In thirteen patients who had been treated with a gluten free diet and replacement therapy, previous loading again had no effect on the excretion of radioactivity. (Table 20). The previously "loaded" treated patients did not differ from the control group ($t=2.012$; $0.5<p<0.01$) but differed significantly from the untreated patients ($t=2.231$; $0.02<p<0.05$). Table 20

Miscellaneous Conditions

The standard oral test dose was given to three

FIGURE 19. Cumulative urinary excretion of radioactivity after 1.0 mgm. of 35S-thiamine hydrochloride (THCL) orally and 200 mgm. non-radioactive thiamine intravenously in a control subject and a patient with primary malabsorptive disease.

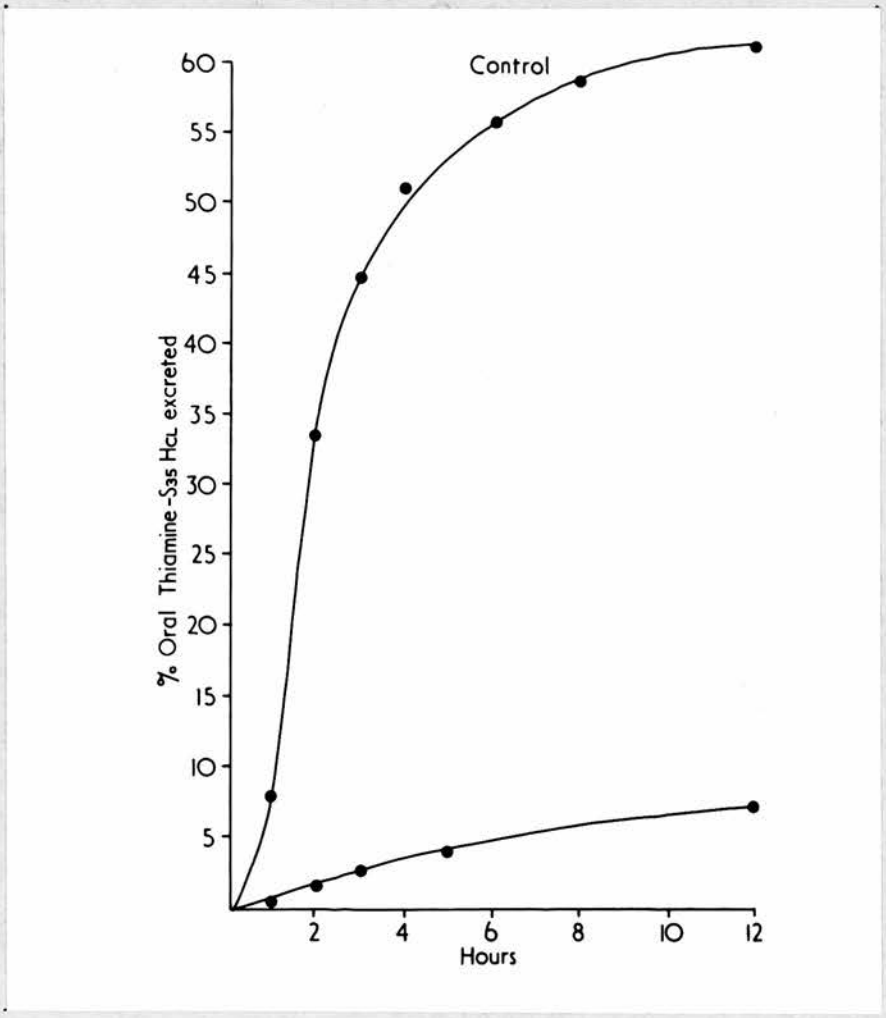

Control
% Oral Thiamine-S35 HCL excreted
60
55
50
45
40
35
30
25
20
15
10
5
2
4
6
8
10
12
Hours

Table 19. The effect of additional flushing doses in a patient with untreated primary malabsorptive disease

The patient was given 300 mgm. thiamine intravenously 48 hours before each test.

Time hr	Urinary radioactivity after Standard Test	Time of additional 100 mgm. i.v. flushing doses of thiamine	Urinary radio-activity after additional flushing
0 - 2	0.3		0.3
2 - 4	7.4		7.0
		4 hr ----------→	
4 - 6	4.8		6.1
		6 hr ----------→	
6 - 12	6.4		10.9
		12 hr ----------→	
12 - 24	6.3		5.0
	25.2		29.3

Table 20. 35S-Thiamine HCl absorption in treated primary malabsorptive disease before and after saturation

Age	Sex	Hb g/100ml	P.C.V. %	Marrow	Serum folate ng/ml	FIGLU	Folic acid absorption	Serum Vit.B_{12} pg/ml	Vit. B_{12} absorption % oral dose	Faecal fat g/day	35S-THIAMINE HCl Urinary Excretion radioactivity (%) Before Saturation	35S-THIAMINE HCl Urinary Excretion radioactivity (%) After Saturation	Duration Gluten Free Diet	Intestinal Mucosa
68	F	9.9	36	Meg.	3.0	+ve	-	239	Normal 16.0%	4.0	17.3	19.1	1 yr.	Subtotal villous atrophy
41	M	14.2	43	Normal	1.6	++ve	Malab.	97	Normal 14.9%	3.7	19.7	19.8	4 mos.	Subtotal villous atrophy
61	F	7.9	17	Meg.	5.5		Normal	-	Normal 33.4%	6.9	9.6	26.8	2 yrs.	Subtotal villous atrophy
57	M	13.9	44	Normal	1.5	+++	Malab.	220	Normal 13.4%	30.7	43.3	33.8	3 mos.	Subtotal villous atrophy
21	F	6.3	22	Meg.	0.9	+ve	Malab.	236	Normal 16.9%	4.9	34.8	38.1	9 mos.	Subtotal villous atrophy
34	M	15.5	45	-	2.0	+ve	Malab.	134	Malab. 3.8%	21.6	28.4	40.1	1.5 yrs.	Partial villous atrophy
41	F	10.7	37	Meg.	2.9	+ve	Malab.	228	Normal 19.6%	8.5	43.3	42.0	1 yr.	Subtotal villous atrophy
58	F	8.7	34	-	-		-	210	Normal 12.1%	15.6	41.0	42.0	2 yrs.	Failed
55	F	8.1	29	Meg.	-		Malab.	179		79.9	38.5	43.5	1 yr.	Subtotal villous atrophy
46	M	12.5	43	-	-		-	-	-	18.2	51.0	49.1	5 yrs.	Subtotal villous atrophy
16	M	14.2	44	-	9.6	-ve	Normal	230	Normal 15.7%	9.5	55.5	53.7	6 mos.	Subtotal villous atrophy

Table 20. CONTINUED

ge	Sex	Hb g/100ml	P.C.V. %	Marrow	Serum folate ng/ml	FIGLU	Folic acid absorption	Serum Vit.B_{12} pg/ml	Vit. B_{12} absorption % oral dose	Faecal fat g/day	35S-THIAMINE HCl Urinary Excretion radioactivity (%) Before Saturation	After Saturation	Duration Gluten Free Diet	Intestinal Mucosa
6	F	9.3	38	Normal	6.7	-ve	Malab.	97	-	4.7	32.5	55.1	2 yrs.	Subtotal villous atrophy
3	M	11.3	41	Normal	2.3	+ve	Malab.	532	Malab.	6.6	64.4	67.0	3 yrs.	Failed
										MEAN	36.9	40.8		
										SEM±	±4.33	±3.84		

Results = at time of diagnosis. Thiamine absorption tested at varying times after treatment.

alab. = malabsorption.

patients with Crohn's Disease (Table 21). The test in patient No. 1 was performed three weeks prior to operation during which 33.5 cm. of terminal ileum were resected and found to have severe changes consistent with a diagnosis of Crohn's Disease. Patient No. 2 had had 50 cm. of terminal ileum resected and a transverse colostomy performed and in patient No. 3, 17 cm. of jejunum had been resected from a point about three feet from the duodeno-jejunal flexure. In both of these patients, the pathologist reported changes consistent with Crohn's disease. The results of the test were normal in all three patients.

Patients with untreated pernicious anaemia also showed normal results (Table 22).

Gastric Surgery

The results in eight patients with gastro-enterostomies are shown in Figure 20 and Table 23. The rate of absorption and the total urinary excretion of radioactivity were normal ($t = 0.395$; $p < 0.5$).

The absorption of thiamine following partial gastrectomy was also studied in eight patients and the results are presented in Table 24. The mean absorption was 70.6% $\pm$ 4.8. When this was compared with the control group using the student t-test, and was not found to be

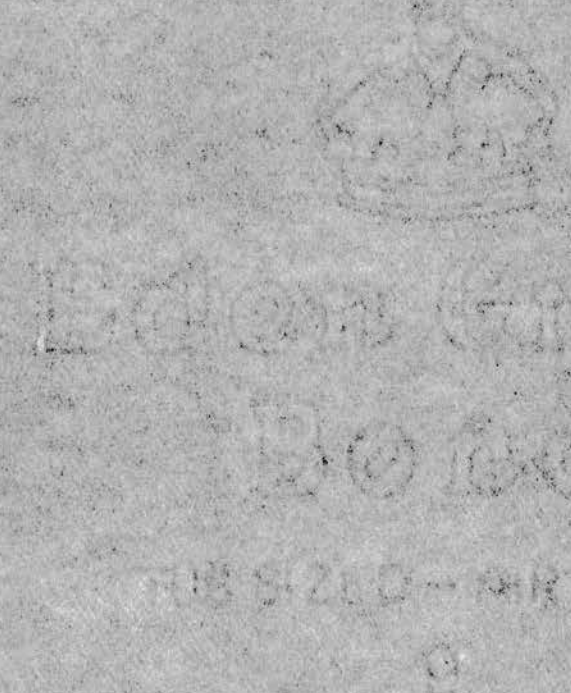
Table 21. Standard oral test in three patients with Crohn's disease previously saturated with 300 mgm. of thiamine intravenously

No.	Age	Sex	Hb gm/100ml	P.C.V. %	Serum folate ngm/ml	Serum Vit. B_{12} pgm/ml	Vit. B_{12} Absorption % oral dose	THIAMINE-35S HCl Urinary Excretion Radio-activity (%)
1.	48	F	14.4	44	6.1	190	Malabsorption 6.3%	35.2
2.	42	F	14.6	43	2.6	207	Equivocal 10.7%	52.0
3.	49	F	13.6	40	4.8	148	Malabsorption 5.09%	60.5

Table 22. The standard oral test in three patients with pernicious anaemia

No.	Age	Sex	Hb gm/100ml	P.C.V. %	Marrow	Serum Vit. B_{12} pgm/ml	Acid Secretion HCl in mEq/l	THIAMINE-35-S HCl Urinary Excretion Radio-activity (%)
1.	70	F	10.4	33	Meg.	105	Achlorhydria	32.8
2.	78	F	7.3	22	Meg.	50	Achlorhydria	42.4
3.	43	M	8.2	24	Meg.	75	Achlorhydria	53.0

FIGURE 20. Thiamine hydrochloride absorption in various clinical states.



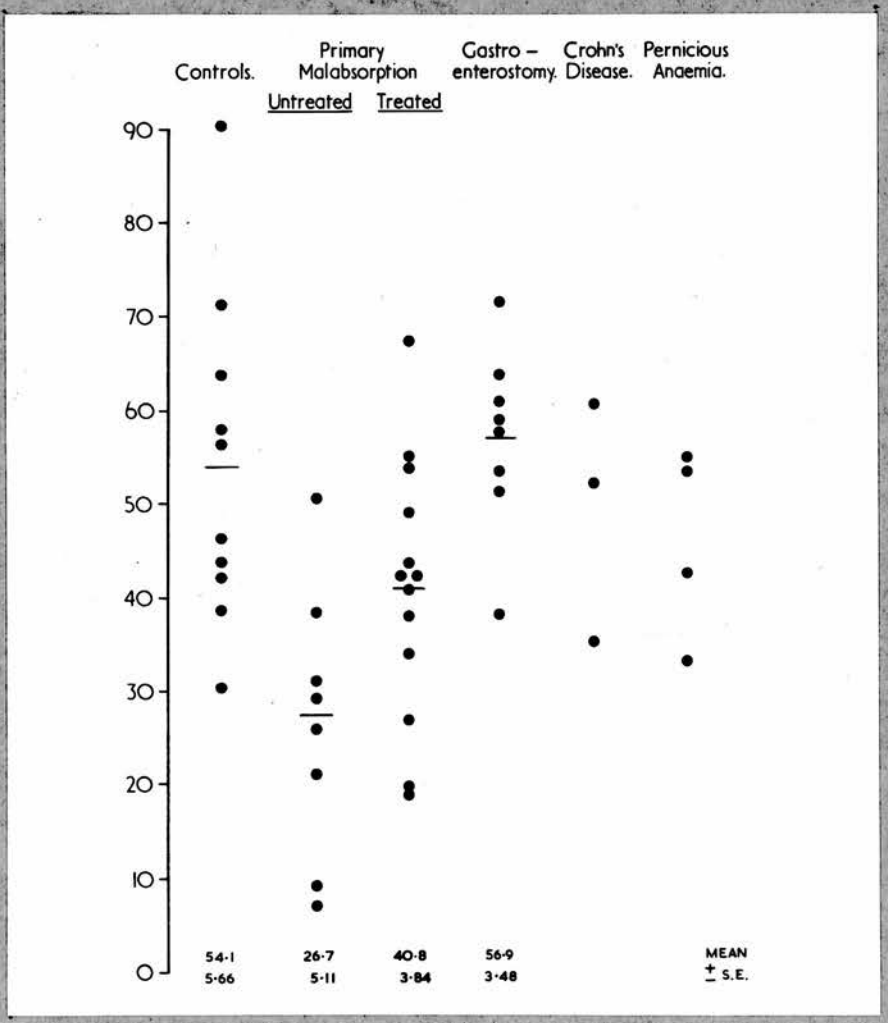

Controls.
Primary Malabsorption
Untreated
Treated
Gastro – enterostomy.
Crohn's Disease.
Pernicious Anaemia.
90
80
70
60
50
40
30
20
10
0
54·1
5·66
26·7
5·11
40·8
3·84
56·9
3·48
MEAN
± S.E.

Table 2.3 Standard oral test in eight patients after gastro-enterostomy

Age	Sex	Hb g/100ml	P.C.V. %	Serum folate ng/ml	FIGLU	Serum Vit. B_{12} pg/m.	Vit. B_{12} absorption %	Faecal fat g/day	Acid Secretion Total HCl in mEq/L	35S-THIAMINE HCl Urinary excretion Radioactivity (%)	Intestinal mucosa	Date of Gastro-enterostomy
70	F	12.4	40	6.3	-ve	441	Equivical	38.2	0.0	38.2	Mild atrophy	1960
56	M	14.6	46	8.0		103	Malab. 2.4%	-	1.25	51.0	-	1957
54	F	13.9	40	8.1	-ve	939	Normal 29.2%	4.1	2.6	53.4	Normal	1960
43	F	12.6	41	2.9	+ve	149	Malab. 7.1%	25.7	0.0	57.1	Normal	1963
51	M	15.0	47	12.9	-ve	429	-	31.8	34.2	59.4	Normal	1959
45	M	15.5	48	-		-	Malab. 4.7%	10.7	-	60.1	-	1960
70	M	14.2	43	7.6	-ve	-	-	-	6.5	64.0	Normal	1960
65	F	15.0	48	4.9	+ve	74	-	17.5	-	71.6	-	1961
										MEAN- 56.9 S.E.+ 3.48		
Normal values:		13.6-16.0		4.5-8.0		< 196	<12.1%	> 5.0				

Malab. = malabsorption.

Table 24. Thiamine absorption in eight patients with partial gastric resections previously saturated with 300 mgm. of non-radioactive thiamine intraveneously

No.	Age	Sex	Hb g/100ml.	P.C.V. %	%Oral 35S-thiamine occurring in Urine 1.0 mgm.	5.0 mgm.	Date Operation
1.	79	M	13.0	40	49.5	43.3	Partial gast. 1960
2.	56	F	13.2	42	52.9	48.3	Partial gast. 1959
3.	73	F	12.5	38	65.6	-	Subtotal gast. 1949
4.	50	F	12.0	39	69.6	61.1	Partial gast. 1954
5.	45	M	11.9	39	71.6	-	Partial gast. 1948
6.	76	F	11.2	38	76.1	-	Partial gast. 1962
7.	66	M	14.0	46	81.4	-	Partial gast. 1957
8.	69	M	13.6	47	98.8	-	Partial gast. 1955
					70.6	50.9	
9.	68	M	13.0	40	3.8	-	Partial gast. 1955

significantly different $t=1.8228$ $p<0.1>0.05$. One patient with a partial gastrectomy excreted only 3.8% of the oral dose. This patient had an abnormal villous pattern of the intestinal mucosa. In some areas the villi were normal in appearance but in others they were broad, interlacing and even fused. The changes were not typical of malabsorption and may have been associated with altered gastric function but, since the findings were exceptional, the patient was not included in the statistical evaluation. He excreted 89% in the first 24 hours when the same dose was given intravenously.

Patient number 3 had had a subtotal proximal gastrectomy with an end-to-side oesophago-gastric anastomosis for an anaplastic carcenoma of the stomach 17 years previously, but there was no postmortem evidence of a recurrence when she died of a myocardinal infaction some months after completion of the studies.

Influence of bacterial flora on thiamine absorption.

The influence of bacterial flora on thiamine absorption was investigated using organisms which had been obtained from the jejunal juice of patients with primary malabsorptive disease and freeze dried. The organisms were kindly donated by Dr. Dellipiani (Dellipiani and Girdwood, 1964). The uptake of cyanocobalamine by the

bacteria was tested and the findings were almost identical to those obtained previously. Their ability to utilize both 35S-thiamine and tritiated H^3-folic acid was tested by adding 477 nc. of folic acid or 49 nc. of 35S-thiamine. The solution was sterilized at 15 lb. pressure for 10 minutes. One ml. of a sixteen hour culture of an organism was added to an aliquot. Two standards of 10 ml. of broth with added labelled vitamin but inoculated with one ml. of sterile saline, were used as controls. After overnight incubation at 37°C the material was centrifuged at 3,000 revolutions per minute for 30 minutes and compared with that in the uninoculated control samples. The radioactivity in the supernatant was calculated using a well-type scintillation counter or Pakard scintillation counter. Measurement of radioactivity in the organisms confirmed that it had been removed from the culture medium and could not be washed off the organisms. The results are shown in Table 25.

The rate of uptake of cyanocobalamine by two organisms shown to utilize vitamin B_{12} was investigated by preparing 6X 10 ml. of broth containing 58 Cobalt-labelled cyanocobalamine as above. Following incubation, a tube was centrifuged after zero time, one hour, two hours, five hours, twelve hours and twenty-four hours and the radioactivity in the supernatant measured. The results are

Table 25. Uptake of labelled cyanocobalamine, folic acid, and thiamine by organisms isolated from patients with malabsorptive disease

Organism	% Uptake of Radioactivity		
	Cyanocobalamine	Folic Acid	Thiamine
	58Co	H3	35S
	0.38nc/ml.	47.7nc/ml.	49nc/ml.
1. Klebsiella (1)	88%	0%	0%
2. Klebsiella (2)	90%	0%	0%
3. Escherichia	0%	0%	0%
4. Escherichia Citrobacter	84%	0%	0%
5. Coliform	92%	0%	0%
6. Streptococcus feacalis	6%	0%	0%

nc = nanocurries

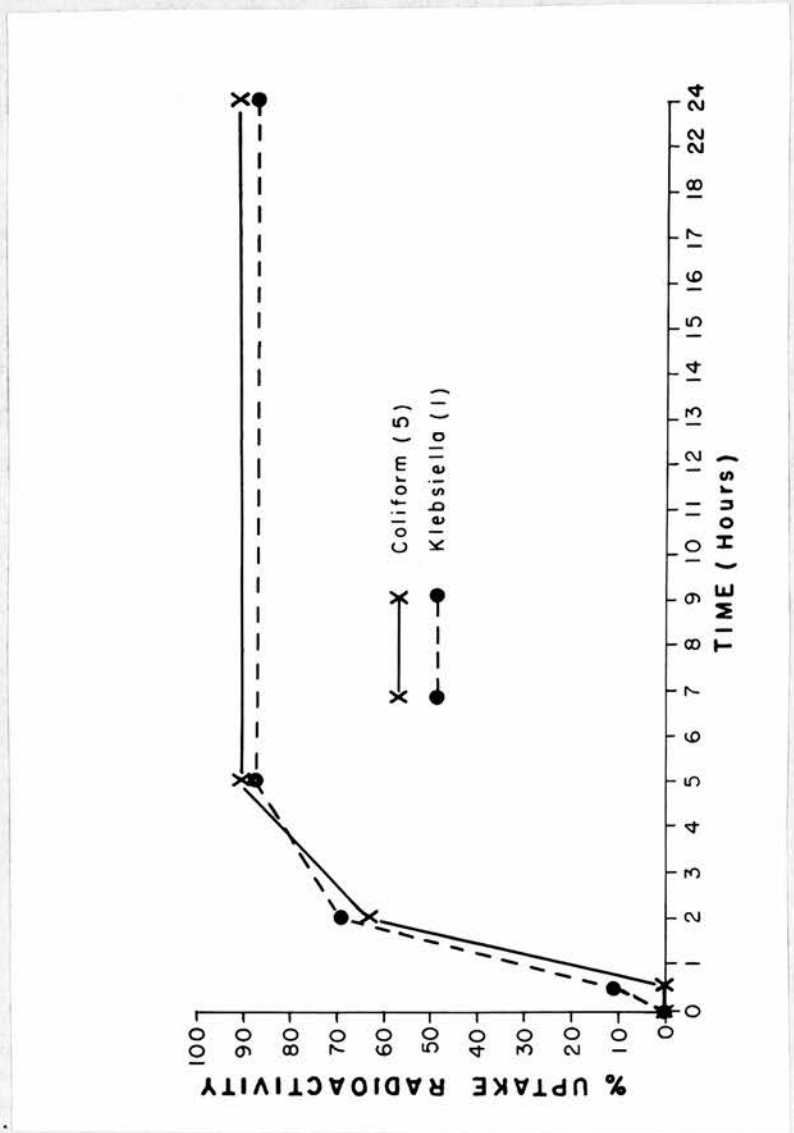
shown in Figure 21.

Although four of the organisms removed over 85% of the cyanocobalamine from the incubation medium, no uptake of thiamine or folic acid could be demonstrated. In view of the rapid absorption of thiamine suggesting that absorption occurs primarily in the jejunum, the absence of faecal flora from the jejunum in most patients as in controls and the failure of the organisms isolated to take up thiamine invitro, it seems unlikely that malabsorption in primary malabsorptive disease is secondary to utilization of the orally administered thiamine by bacteria. This conclusion is in keeping with the previous findings that little breakdown of thiamine appears to occur in the lumen of the intestine.

DISCUSSION

Patients with untreated primary malabsorptive disease excreted significantly less radioactivity than the control group ($t = 3.504$ $p < 0.01$). The cumulative urinary excretion showed delay and marked reduction in the total excreted. These factors were uninfluenced by previous loading with 300 mgm. of thiamine intravenously or by additional flushing doses given during the 0-48 hour period. Using these tests, I was able to exclude the possibilities that the flushing dose was inadequate or

FIGURE 21. The rate of uptake of 58C. cyanocobalamine by organisms isolated from patients with malabsorptive disease.


% UPTAKE RADIOACTIVITY
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 22 24
TIME (Hours)
Coliform (5)
Klebsiella (1)

that absorption had occurred after the flushing dose had been excreted. The difference between the patients with malabsorption and the controls was not due to a decreased ability to excrete thiamine as a patient was able to excrete an intravenous dose normally.

At the time that this part of the work was being carried out, the restrictions on the amount of 35S-thiamine which could be given to each patient made it necessary to test absorption at one dose level only. It was decided to choose a physiological dose which would be large enough to show that the subject was capable of absorbing his minimum daily requirement during the 24 hours but which did not exceed the reserve capacity of the intestine in the control subject. The minimum daily requirement in the adult human has been estimated at between 0.23 mgm/1000 cal and 0.66 mgm/1000 cal (Williams, et al., 1942; Oldham, et al., 1944; Daum, et al., 1949; Dick, et al., 1958 and Ziporin, et al., 1965). The work of Morrison & Campbell (1960); Friedemann, et al., (1948) and Schultz, et al., (1938) suggests that little further absorption occurs when the dose of thiamine exceeds 4-5 mgm. Therefore, 1.0 mgm. was chosen as a reasonable oral dose.

The paired t-test showed no significant difference between malabsorptive patients who had been previously

loaded and those who had not, either before, or after treatment. It was also shown that the mean of the untreated malabsorptive patients, with previous "loading" was significantly less than the treated patients. However, the mean of the treated malabsorptive patients, who were not given 300 mgm. 48 hours before treatment, was low. Consequently, further statistical tests were applied. Seven of the eight differences in the untreated patients were positive but the result of the Wilcoxen's sign test (Spiegel, 1956) confirmed the t-test findings. Similarly, in control subjects, previous "loading" did not significantly alter the result. Consequently, the average of the result before and after "loading" was taken in each malabsorptive patient and the resulting two groups compared with the control group considered as twenty independent observations. A one-way analysis of variance on these three groups was found to be highly significant confirming the difference between the untreated and control groups. $P[F \pm 5.21;\ d.f.\ (2,38)] = 0.99$. The one-tailed t-test applied to the average of the treated and untreated malabsorptive patients, gave $t = 1.8$ with 19 d.f. and this was significant at the five per cent level.

Although thiamine absorption primarily seems to occur high in the gastro-intestinal tract, it is possible that, in the patient with primary malabsorptive disease, more absorption occurs in the ileum. However, most of the

thiamine is absorbed during the first 4-5 hours even in these patients and for bacteria to significantly reduce the amount of thiamine available, they would need to be present in sufficient numbers at a level where absorption is occurring. None of the bacteria isolated from the jejunum or the ileum of patients with primary malabsorptive disease could be shown to utilize thiamine when incubated invitro for 24 hours. The possibility that the reduction in absorption is only apparent and that thiamine is being produced, by abnormal bacteria, in quantities adequate to give reduced absorption of the orally administered thiamine, is very doubtful. Thiamine deficiency can be corrected by refected rat faeces (Mickelson, 1956), and liver thiamine levels are higher in conventional than germ-free chicks given a thiamine free diet (Luchey et al., 1955). Nevertheless the nutritional importance of intestinally synthesised thiamine must be questioned since the thiamine sparing effect of antibiotics is negated by prevention of coprophagy (Marneesh et al., 1959; Barnes et al., 1960). Since thiamine synthesised by micro-organisms appears to exist in a macromolecular form that is not well absorbed (Wostmann and Knight, 1961), and since even free thiamine is poorly absorbed from the caecum (Kwong et al., 1962).

Consequently, there is little evidence that abnormal

bacteria play a significant role in the malabsorption of the patient with primary malabsorptive disease. It is also true that thiamine deficiency per se cannot be the cause of these patients failing to absorb thiamine since intravenous thiamine did not improve absorption. Neither was there any correlation between the ability of these patients to absorb thiamine and other intestinal absorption tests or with the severity of the changes shown by jejunal biopsy. The results in these patients illustrate again the variation in the ability of individual patients to absorb different substances during the acute phase of the disease.

Wang & Harris (1939) and Brummer & Markkanen (1960) measured the daily urinary excretion of dietary thiamine in achlorhydric subjects and found evidence of reduced absorption. During the present investigation, four patients with pernicious anaemia and three with histamine fast achlorhydria following gastric surgery, were all found to excrete normal amounts of radioactivity. If absorption is grossly impaired, malabsorption may become apparent when the test dose is small. A larger oral dose, however, may result in a greater difference between normals and patients or may demonstrate impairment which was not obvious with the smaller dose as occurs with xylose absorption. Consequently, the results in

achlorhydria are not necessarily incompatible with the observations of Wang & Harris (1939) and Brummer & Markkanen (1960) although there may be other factors producing depletion in their patients.

Three patients with Crohn's disease involving the terminal ileum, and showing impaired absorption of vitamin B_{12}, excreted normal amounts of radioactivity after the standard test for thiamine, suggesting that absorption of vitabine B_1 in the human is mainly in the upper small intestine.

In eight patients who had had gastro-enterostomies, there was no alteration in either the rate of absorption or in the total urinary excretion of radioactivity. Eight patients with partial gastrectomies also failed to show any significant difference from the control group. One patient with a partial gastrectomy excreted only 3.8% of the oral dose and was excluded from the statistical evaluation. This patient had an abnormal villous pattern which may have been associated with altered gastric function. He excreted 89% in the first 24 hours when the same dose was given intravenously. A patient who had had a sub-total gastrectomy 17 years previously excreted 65.5%. The presence of a normally functioning stomach does not appear to be essential for the absorption of physiological amounts of thiamine.

CHAPTER III

STUDIES IN OLD AGE

Introduction

Evidence of thiamine deficiency has recently been reported in elderly subjects by several workers. (Brin, etal., 1964; Brin et al., 1965; Griffiths, et al., 1965). Intestinal absorption has an important role in determining the nutritional status but has not been extensively studied in old age. This problem is of particular interest because of reports of structural changes in the small intestine in later life (Lascalea, 1959; Andrew, 1961; Suntzeff & Argeletti, 1961) and the demonstration by Fry et al.,(1961) of a reduced rate of cell production in the crypts of the jejunum of mice.

There is evidence of impaired absorption of fat (Becker, et al., 1950), amino acids (Wild, et al., 1953), galactose (Meyer et al., 1943) and of certain vitamins - vitamin A (Yiengst & Shock, 1949) and vitamin B_{12} (Chow, 1954; Glass, et al., 1955). Although Chinn, et al. (1956) found no significant difference in the rate or the extent of protein digestion and absorption in old age and the findings of impaired absorption of vitamin B_{12} have not

been confirmed by subsequent work (Swendseid, et al., 1954; Chow, et al., 1956; Tauber, et al., 1957; Hyams, 1964).

Rajsky & Newman (1943) measured the urinary excretion of thiamine in twenty-two elderly subjects and found that a larger dose was required to produce a constant output than in younger subjects. This may have resulted from reduced absorption although there is evidence that suggests thiamine requirement increaseswith age (Mills, 1948; Oldham, 1962). The work of Draper (1958) in the rat showed that the absorption of an oral dose of radiothiamine decreased substantially beyond the age of 20 months. Kirk & Chieffi (1951) measuring faecal thiamine in man, however, found little change in percentage absorption with increasing age at the dose level used.

Methods and Subjects Studied:

Method for the study of absorption of 35S-thiamine.

The radioactive material was diluted with non-radioactive thiamine so that each test dose contained 1.0 mgm., 5.0 mgm. or 20.0 mgm. and 5μc of radioactivity dissolved in 20 ml. of water. The test dose was given orally to subjects after an overnight fast and a parenteral injection of 200 mgm. of non-radioactive thiamine was given immediately before the oral dose. Urine collections were removed

after 5 hours, 12 hours, 24 hours and 48 hours and counted as previously described.

The effect of additional flushing doses in elderly subjects

Three subjects over the age of eighty were given 20.0 mgm. of radioactive thiamine orally and 200 mgm. flushing dose. The results were compared with those obtained when the test was repeated in the same subject with additional 100 mgm. flushing doses given intravenously at 4 hours, 9 hours and 24 hours after the oral dose. Urine was collected at hourly intervals for the first four hours, then at 12 hours, 24 hours, 36 hours and 48 hours. The details of a 90 year old subject are shown in Table 26. The results obtained in the other two subjects were 21.0%, 22.7% and 17.7%, 16.3%. These results are similar to those obtained in younger patients and consequently the 200 mgm. flushing dose was subsequently used at all three dose levels.

Subjects Studied.

Two groups of convalescent hospital in-patients who were free from haematological, gastro-intestinal, endocrine or malignant disease, were studied: Group 1 consisted of 24 subjects aged 76 - 90 years (mean 82.1 years). Group II consisted of 21 younger subjects aged from 28 - 56 years (mean 48.9 years). Fifteen members of the older

Table 26. Effect of additional flushing doses in a ninety year old subject

Time HR	Urinary radioactivity* after first test**	Time of additional 100 mgm. i.v. flushing doses of thiamine	Urinary radioacitivity after additional flushing
0 - 1	0.45		0.59
1 - 2	0.57		0.19
2 - 3	2.45		3.29
3 - 4	1.57		1.49
4 - 12	6.31	4 hr.	6.65
12 - 24	7.06	9 hr.	4.96
Total for 24 hr.	18.41		17.17
		24 hr.	
24 - 36	2.53		2.93
36 - 48	2.58		4.14
Total for 48 hr.	23.52		24.24

*Result expressed as percentage oral dose.
Subject given 20 mgm. radioactive thiamine orally.

**First test = 20.0 mgm. radioactive thiamine orally and 200 mgm. non-radioactive flushing dose intravenously at the same time.

group received a test at each of the oral dose levels i.e. 1.0 mgm., 5.0 mgm., and 20.0 mgm. of thiamine. The order in which the tests were performed was randomised. The remaining nine were given one test only. Ethical reasons prevented more than two tests being carried out on any individual in the younger group. The dose to be omitted was again determined from random number tables (Documenta Geigy, 1965).

RESULTS

The results obtained when subjects in both groups were given tests at each of the three oral dose levels are summarised in Tables 27, 28, and 29. In figures 22, 23, and 24 the percentage excretion of radioactivity of both the younger and the older groups, at each dose level, have been plotted against age. A regression analysis was performed at each of the dose levels but no correlation between age and the percentage excretion of radioactivity could be demonstrated [At 1.0 mgm. oral dose/age $t = \pm 0.002$ (35 d.f.) $p > 0.5$; 5.0 mgm. $t = \pm 0.687$ (27 d.f.) $p = 0.5$; 20.0 mgm. $t = \pm 0.673$ (30 d.f.) $p > 0.50$].

Rate of excretion of the flushing dose and orally administered thiamine in old age.

Attempts to assess the rate of thiamine absorption in elderly subjects were often difficult to interpret because of frequent renal impairment and retention of urine in the

Table 27. Thiamine absorption in younger and older groups at three oral dose levels

Oral dose radioactive thiamine mgm.	Urinary Excretion of radioactivity % oral dose* OLDER	YOUNGER
1.0	53.1 ± 3.03 (21)	51.8 ± 2.97 (16)
5.0	33.4 ± 2.14 (17)	40.8 ± 4.71 (12)
20.0	21.2 ± 1.23 (18)	25.8 ± 3.69 (14)

*The results are expressed as the mean ± SEM (n). Urine was collected for 48 hours.

Subjects were given 200 mgm. non-radioactive thiamine flushing dose intravenously at the time of the oral dose.

Table 28. Thiamine absorption in the younger group at three dose levels

No.	Age	1.0 mgm. Oral Dose	5.0 mgm. Oral Dose	20.0 mgm. Oral Dose
1	28	66.0	-	17.4
2	30	-	31.2	25.2
3	45	-	38.6	39.8
4	45	40.0	-	36.6
5	46	45.3	-	19.1
6	48	58.0	29.1	-
7	48	51.2	40.1	-
8	49	-	72.1	8.5
9	50	36.1	-	24.8
10	50	31.2	-	22.9
11	51	63.6	-	50.0
12	51	64.6	18.4	-
13	51	46.2	54.3	-
14	52	37.8	-	16.2
15	52	59.8	-	23.6
16	53	-	22.3	16.1
17	53	66.9	-	52.6
18	53	45.7	38.7	-
19	54	51.6	38.4	-
20	55	-	38.1	9.2
21	56	65.5	68.2	-
MEAN		51.8	40.8	25.8
± SEM		2.97	4.71	3.69

Results are expressed as % radioactive oral dose excreted.

Subjects were given 200 mgm. non-radioactive thiamine flushing dose intravenously at the time of the oral dose.

Urine was collected for 48 hours.

Table 29. Thiamine absorption in the older group at three oral dose levels

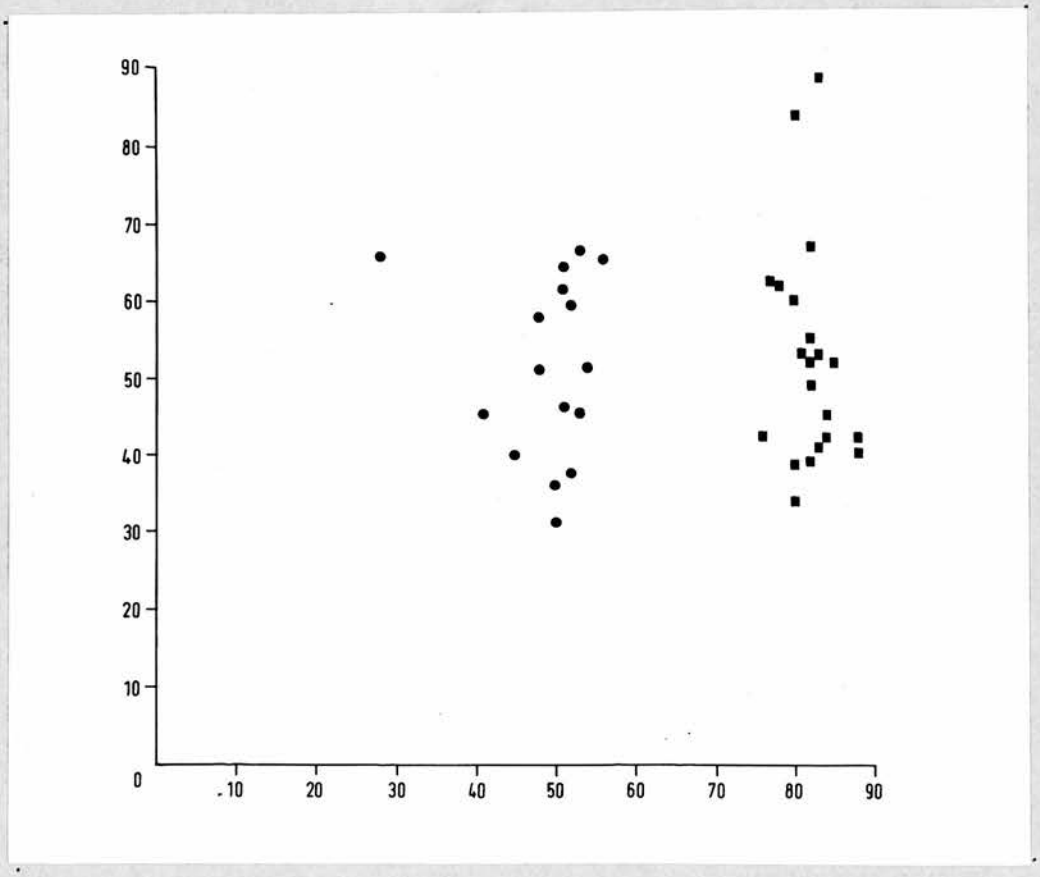
Age	Sex	1.0 mgm. Oral Dose	5.0 mgm. Oral Dose	20.0 mgm. Oral Dose
76	M	42.6	32.0	18.0
77	F	62.9	52.5	28.5
78	F	62.6	41.3	17.4
80	F	38.8	43.9	20.8
80	F	60.0	32.5	22.4
81	F	53.1	25.9	21.0
82	F	39.5	38.7	25.5
82	F	55.1	23.6	27.9
82	M	49.0	36.5	22.6
83	F	41.2	26.0	14.9
84	M	45.2	26.0	16.5
84	M	42.5	41.2	21.2
85	M	52.3	24.5	13.4
88	F	40.7	18.0	26.5
88	F	42.3	32.1	15.6
77	M	-	-	18.1
80	M	84.0	-	-
80	M	34.1	-	-
82	M	52.3	-	-
82	F	67.2	-	-
83	F	53.0	-	-
84	M	-	37.8	-
90	M	-	-	23.5
MEAN ± SEM		53.1 3.03	33.4 2.14	21.2 1.23

Results are expressed as % radioactive oral dose excreted.

Subjects were given 200 mgm. non-radioactive thiamine flushing dose intravenously at the time of the oral dose.

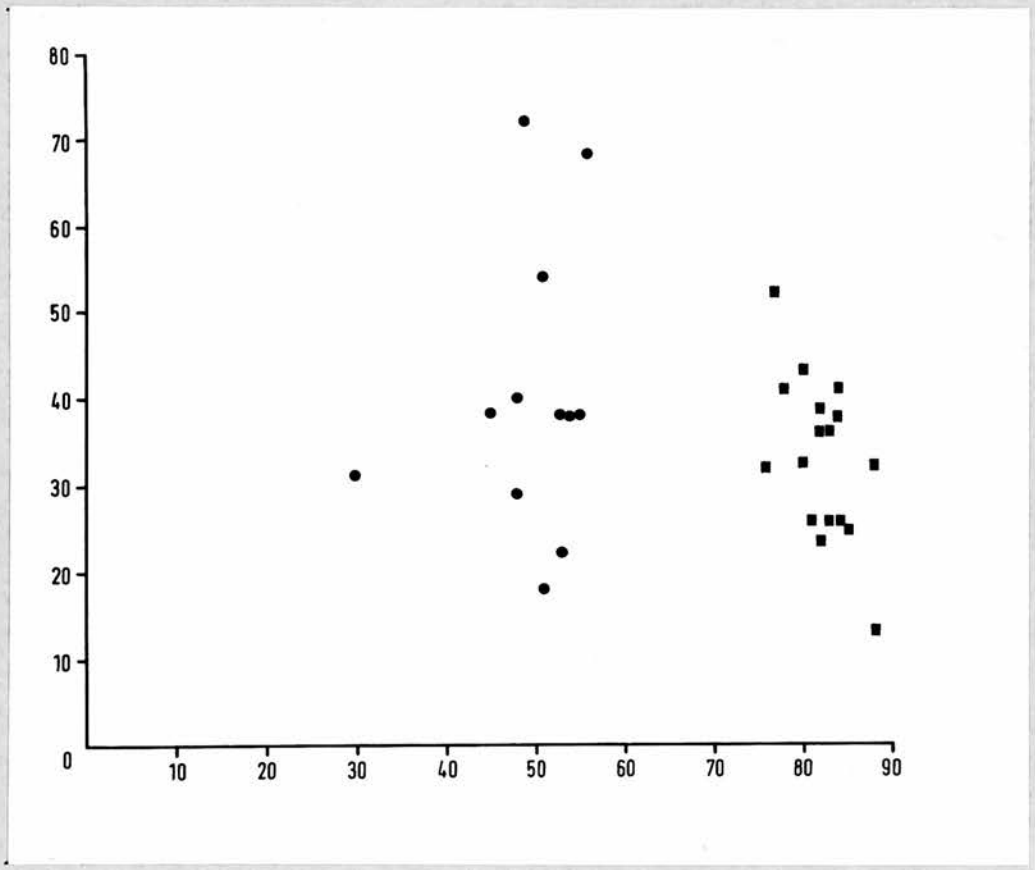
Urine was collected for 48 hours.

FIGURE 22. Excretion of 1.0 mgm. oral dose of 35S-thiamine hydrochloride (THCl) at different ages.



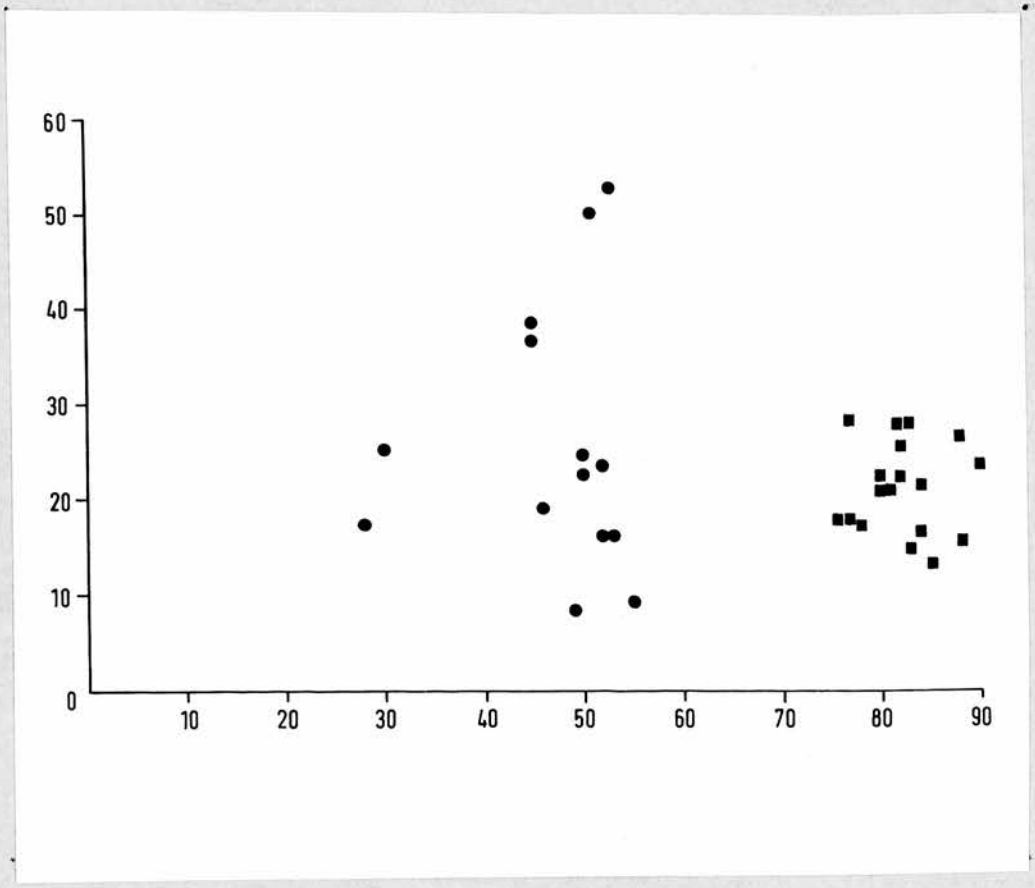
EXCRETION OF 1.0 MGM. ORAL DOSE 35S-THIAMINE
AT DIFFERENT AGES

FIGURE 23. Excretion of 5.0 mgm. oral dose of 35S-thiamine hydrochloride (THCl) at different ages.



EXCRETION OF 5.0 MGM. ORAL DOSE 35S-THIAMINE
AT DIFFERENT AGES

FIGURE 24. Excretion of 20 mgm. oral dose of 35S-thiamine hydrochloride (THCl) at different ages.



EXCRETION OF 20.0 mgm. oral dose 35S-THIAMINE
AT DIFFERENT AGES

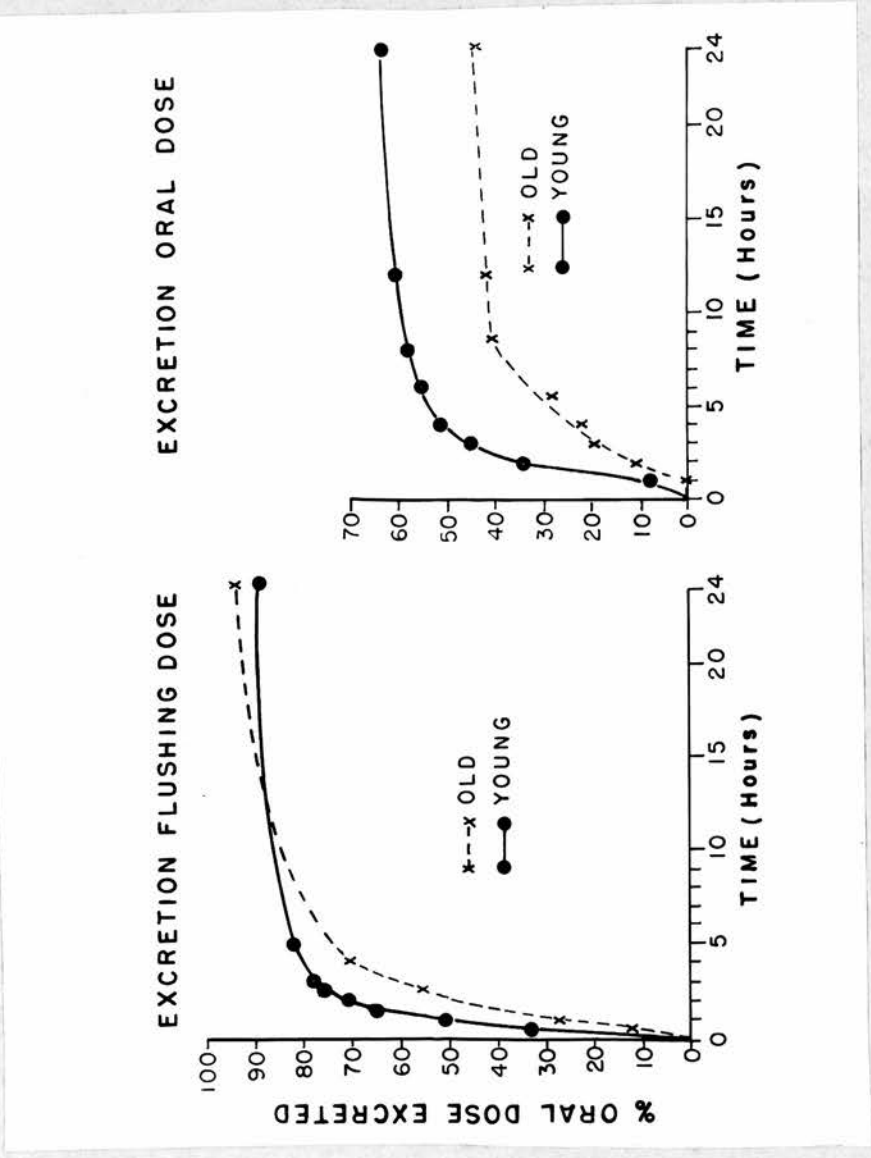
bladder after voiding. Four subjects were chosen who had previously been catheterised but who retained good renal function. The rate of excretion of 200 mgm. thiamine flushing dose was studied by incorporating 35S-thiamine. After one week, 1.0 mgm. of 35S-thiamine was given orally together with a non-radioactive 200 mgm. flushing dose and a divided urine again collected.

The results of one 83 year old man are shown together with those of a young subject in Figure 25. The rate of excretion of the flushing dose was not very much less than in the younger subject but the rate of excretion of the oral dose is significantly reduced. This may be due in part to reduced renal function but it suggests that absorption from the intestine may be slower although the total amount absorbed will not be significantly different from the younger subjects as previously shown.

DISCUSSION

The results agree with those of Kirk & Chieffi (1951). They measured faecal thiamine using a chemical method (Hennessy & Cerecedo, 1939; Friedmann & Kmieciak, 1943) before, during, and after a 5.0 mgm. daily, oral supplement but found little change in the percentage absorption with increasing age. The minimum daily requirement of thiamine for the young adult has been estimated at between 0.23 mgm/1000 cal and 0.66 mgm/1000 cal (as shown in the

FIGURE 25. Comparison of the cummulative urinary excretion of a 35S-thiamine labelled 200 mgm. flushing dose and a 1.0 mgm. standard oral test in a young and old subject.


EXCRETION FLUSHING DOSE
EXCRETION ORAL DOSE
% ORAL DOSE EXCRETED
TIME (Hours)
TIME (Hours)
x--x OLD
● YOUNG
x--x OLD
● YOUNG

discussion of Chapter I of this thesis) and the daily allowance suggested by the National Research Council is 1.3 mgm. per day. (National Research Council Publication, 1958) Evidence suggests that for the rat (Mills, 1948) and for man (Oldham, 1962) this requirement may be increased in old age. However, both at a physiological level and a very much larger doses, no difference has been found between the two groups in this present study. This is not to say that deficiency may not occur due to inadequate intake of thiamine. Despite an apparently adequate diet, the availability of nutrients at the cellular level may vary widely between individuals due to lack of appetite or poor dentition. Food served in institutions may receive extra cooking to make the food tender and readily digested and this may be responsible for destruction of heat labile nutrients. The individuals studied were chosen to exclude certain diseases and nothing can be said about the absorption of thiamine in these patients. Nor were any subjects studied who had advanced protein malnutrition in which severe intestinal atrophy has been observed (Passmore, 1947-1948; Gillman and Gillman, 1951; Brock, 1961; Deo and Ramalingaswami; 1964). However, the findings do suggest that there is not an inevitable decline with age in the ability of the intestine to absorb vitamin B_1 and that deficiency in most elderly people need not arise provided that adequate amounts are present in the diet.

CHAPTER IV

STUDIES ON THE MECHANISM OF THIAMINE ABSORPTION IN MAN

Introduction

There is evidence suggesting that thiamine behaves differently to most other water soluable vitamins and that there is a limit to the amount of thiamine that can be absorbed from a single oral dose. Schultz et al., 1938, state that in the human subject receiving 5.0 mgm. of thiamine daily, almost all of an additional 5.0 mgm. oral dose could be recovered from the faeces. Melnick et al., 1945, found a break in the linear dose-response curve at an intake of approximately 5.0 mgm. daily and Friedmann et al., 1948, concluded that the maximum amount that could be taken orally without resultant increase in faecal thiamine was about 5.0 mgm. per day. These findings were supported by Morrison and Campbell, 1960, who found little increase in the amount of thiamine excreted in a 24 hour urine occurred when an oral dose greater than 2.5 mgm. was given.

The evidence indicating an upper limit to the amount that could be absorbed from a single dose suggests that a saturable mechanism for thiamine absorption may exist in the intestine. Work by Polin et al., 1963a, using an invivo technique in white Leghorn hens indicated that thiamine

absorption from an isolated duodenal loop conformed to the Michaelis-Menten kinetics and that amprolium (an anti-metabolite of thiamine) competitively inhibited thiamine absorption. Further work by the same authors Polin et al., 1963b, in 3 - 5 week old chicks, however, suggested that absorption occurred by diffusion. This is in contrast to the findings of Magyar and Gabor, 1949, who found that prior administration of thiamine reduced the amount of thiamine that could be absorbed from an isolated intestinal loop and to those of Ventura et al., 1963, who showed that comparatively greater absorption of small than large doses of thiamine.

Thiamine absorption using invitro techniques was investigated in rats by Turner and Hughes, 1962, and by Spencer and Bow, 1964. Neither author was able to demonstrate passage of thiamine against a concentration gradient and no reduction in the presence of inhibitors has noted. Ventura and Rindi, 1965, suggested that the two previous workers had chosen an initial concentration which was too high and repeated the experiments using initial concentrations of 0.21μM/l. on both sides of the sac. They concluded that net transport of thiamine against a concentration gradient occurred, that metabolic inhibitors, thiamine analogue pyrithiamine and the decreased incubation temperature inhibited transport suggesting that thiamine was

absorbed by active transport. Sharma and Quastel, 1965, suggested that thiamine transfer into the brain cell of the rat was a carrier mediated mechanism dependent upon sodium pump.

The possibility that thiamine was phosphorylated during its intestinal absorption was suggested by Linneweh and Muller, 1940; and Magyar and Gabor, 1949. Rindi and Ventura, 1966, found a significant increase in phosphorylated thiamine in the intestinal wall two hours after the introduction of thiamine hydrochloride but the relationship between thiamine phosphorylation and thiamine absorption was not established. More recently, Gassmann and Sandner, 1967, proposed that thiamine was phosphorylated in the lumen of the intestine by pancreatic secretions prior to absorption but the experiments left many unanswered questions.

The results obtained by different workers provide conflicting views of the mechanism of thiamine absorption. In man, workers seem to agree that there appears to be a limit to the amount of thiamine that can be absorbed from a single oral dose but the reasons for this have not been identified. The methods used to measure thiamine often fail to identify its many metabolites. The reduction in thiamine excretion secondary to storage and utilization is not known nor was the possibility of thiamine breakdown in the intestine investigated.

In the investigations which follow, the relationship between the oral dose given and the amount of thiamine absorbed in control subjects was studied in an attempt to clarify the factors operative in normal man. The effect of massive intestinal resection on thiamine absorption will also be presented.

RESULTS

Maximum 72 hour urinary excretion of radioactivity following a single oral dose of 35S-thiamine hydrochloride

Fifty-six normal subjects were given oral doses of thiamine hydrochloride ranging from 1.0 mgm. to 20.0 mgm. containing 10 μc. of 35S-thiamine together with 200 mgm. intravenous flushing dose. Each subject was given a test at three oral dose levels, the order in which the tests were performed being randomized. Seven other control subjects were given oral doses of 50 mgm. or 200 mgm. of radioactive thiamine together with a flushing dose. Urine was collected in 0 to 5, 5 to 12, 12 to 24, 24 to 48 and 48 to 72 hour aliquots.

The results obtained are summarised in Table 30. These results are shown plotted in Fig. 26 where the abscissae represent reciprocals of the oral dose of thiamine (d) and the ordinates reciprocals of the total amount of the oral dose excreted in 72 hours (S).

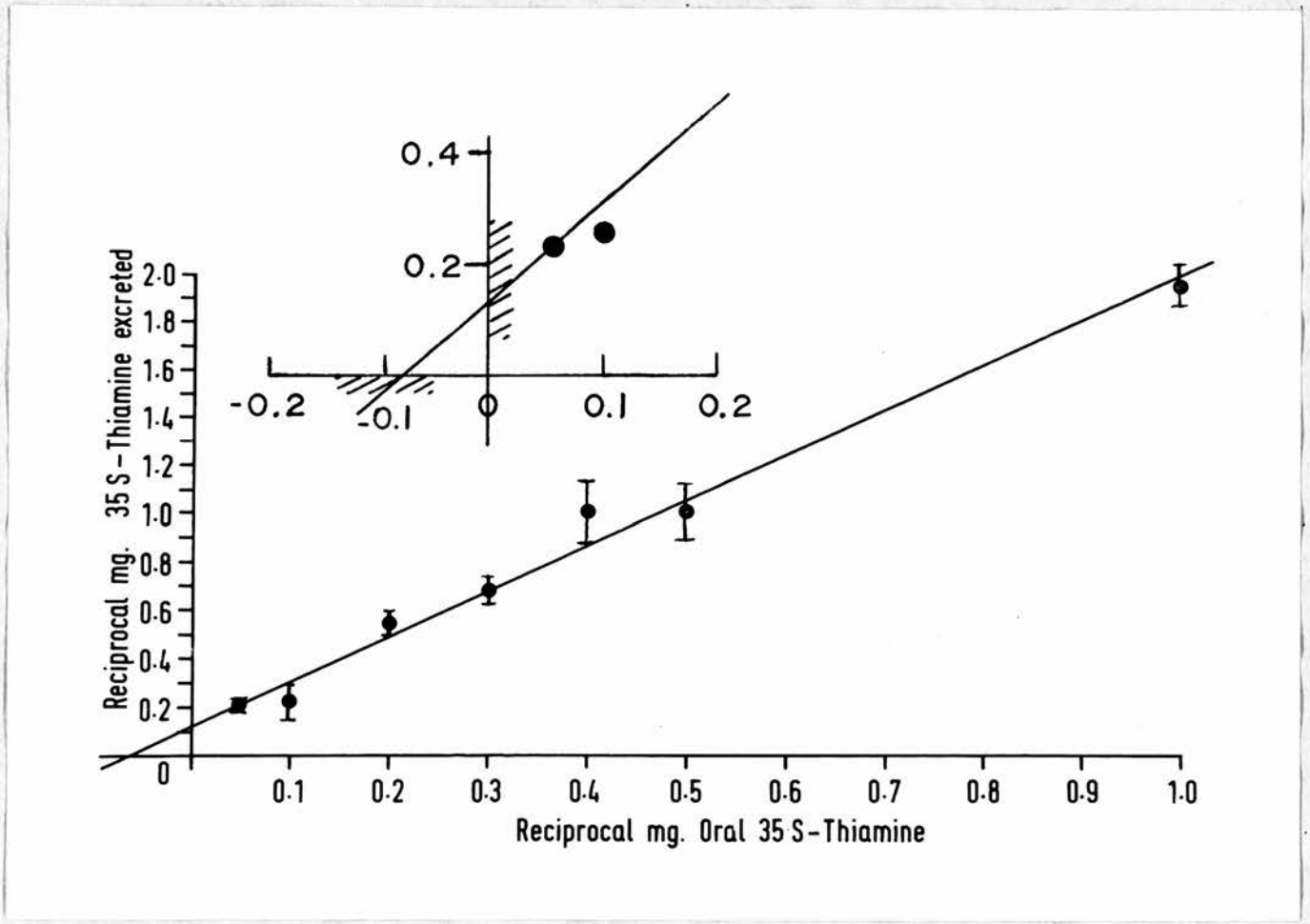
Table 30. Seventy-two hour urinary excretion of radioactive thiamine by normal subjects after varying oral doses of 35S-thiamine hydrochloride*

Oral dose radioactive thiamine (mgm.)	Urinary excretion Radioactivity Per Cent oral dose*	Mean weight** (mgm.)	Number of subjects
1.0	51.0 ± 2.07	0.51 ± 0.02	45
2.0	46.7 ± 4.47	0.93 ± 0.09	10
2.5	39.2 ± 5.07	0.98 ± 0.12	10
3.3	44.4 ± 3.76	1.47 ± 0.12	10
5.0	35.5 ± 1.77	1.77 ± 0.09	45
10.0	43.3 ± 6.63	4.33 ± 0.66	10
20.0	23.8 ± 1.51	4.77 ± 0.36	40
50.0	11.3 ± 1.81	5.63 ± 0.90	5
200.0	3.7 ± 2.01	7.32 ± 4.02	5

*Subjects were given 200 mgm. of thiamine hydrochloride intravenously at the time of the oral dose.

**Results are expressed as the mean ± SEM. Urine was collected for 72 hours.

FIGURE 26. The linear relationship between the reciprocal of the dose of radioactive thiamine (THCl) given orally and the reciprocal of the cumulative 72 hour urinary radioactivity. Each point represents a mean value ± one S.E.; 200 mgm. of non-radioactive thiamine was given intravenously with each oral dose. The inset shows the values of Vmax and Km ± two standard errors.


0.4
0.2
-0.2
-0.1
0
0.1
0.2
Reciprocal mg. 35 S-Thiamine excreted
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
Reciprocal mg. Oral 35 S-Thiamine

Each point has been shown ± one standard deviation. The points fall on a straight line suggesting that the relationship between the oral dose of thiamine administered (d) and the total amount of the oral dose excreted (S) may be represented empirically by an equation of the Michaelis and Menten (1913) type, namely

$$S = \frac{V.d}{K + d} \qquad (1)$$

where,

V = the maximum attainable 72 hour excretion of orally administered thiamine following a single dose.

K = the dose of oral thiamine which gives 72 hour excretion equal to V/2.

This can be transformed to the linear relationship between reciprocals, as in Fig. 25.

$$\left(\frac{I}{S}\right) = \frac{K}{V} \cdot \left(\frac{I}{d}\right) + \frac{I}{V} \qquad (2)$$

Regression analysis using the weighted-least-squares procedure (Wilkinson, 1961) was performed to test the applicability of this formula to the data and obtain V max and the Michaelis constant K. d. was treated as the independent variable and V as a normal randomly distributed variable with the expected value

$$E\ (V/d) = \frac{Vmax.d}{K + d} \qquad (3)$$

and variance

$$\text{Var } (V/d) = \sigma^2 \qquad (4)$$

Calculations of K and V with their standard errors were based on fitting a bilinear regression of V on the corresponding values of the provisionally fitted Michaelis-Menten function and its first derivative (Wilkinson, 1961). Results obtained with doses of 50 mgm. and 100 mgm. were not used in the calculations because of the small number of subjects studied with this dosage. The techniques employed do not permit a direct estimate of the initial rate of absorption but it has been shown by Fisher and Parsons (1953) that if the relationship between the concentration at any moment and the rate of absorption at that moment is of the form of equ.(1), then the relationship between the initial concentration and the average rate of absorption will be expected to be also of the form of equ.(1). As previously discussed in Chapter I, the available evidence suggests that the net passage of thiamine from the intestine is unidirectional and little parenterally administered thiamine appears in the faeces.

When the data was plotted according to the Linweaver-Burk method (1934) a straight line relationship was obtained between administered and excreted radioactive thiamine (Vmax 8.3:K= 12.5). The calculated maximum amount of thiamine (Vmax) absorbed following receipt of a single oral dose (K) required to produce half maximum absorption was 12.0 $\pm$ 2.4 mgm. (range 3.5 - 13.1; confidence probability 0.95). and the

size of the oral dose (K) required to produce half maximum absorption was 12.0 ± 2.4 mgm. These values ± two standard errors have been indicated on the inset of Fig. 26.

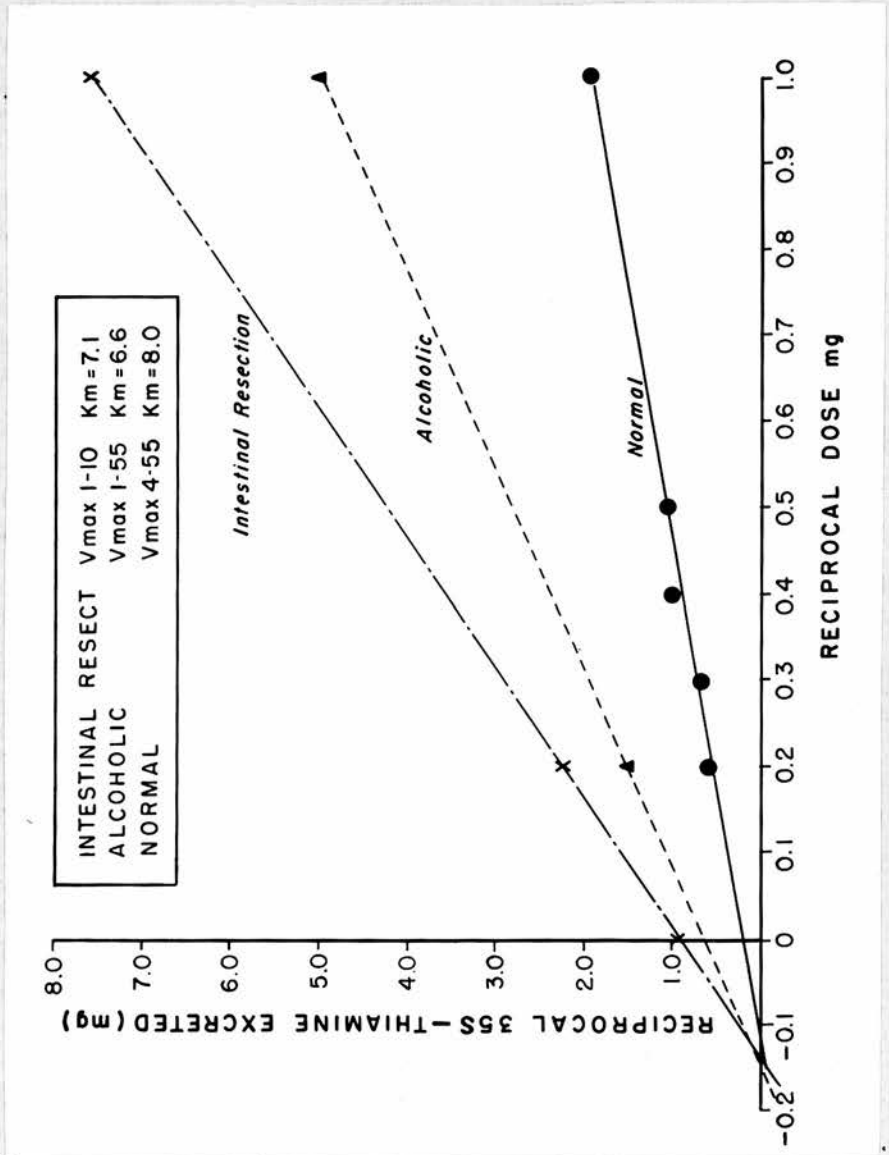
The results must be interpreted with caution. It should be pointed out that the V max. presented is analogous to but not identical with the maximum calculated rate at time zero. A better estimate might have been obtained by using the thiamine excreted during the first 30 minutes after the oral dose but it was decided not to do this because the errors introduced by gastric emptying and passage along the intestine. Similarly, Michaelis-Menten theory is based on changes in concentration of the substrate. It has been assumed that the variation in the amount of fluid present in the intestine with time and between individuals is small but this has not been measured. Since the measurement of thiamine absorption is indirect, it is also possible that the rate limiting factor may not be in the intestinal mucosa but in the transport system of the blood, in the tissues or imposed by renal excretion. These possibilities and additional considerations are discussed later. The present estimate of the amount of thiamine that can be absorbed requires refinement by increasing the sample size.

Influence of intestinal resection on thiamine absorption.

If thiamine is absorbed by a mechanism which can be saturated, then a man who has had an intestinal resection may show a reduction in the maximum amount of thiamine which can be

absorbed from a single oral dose. It may also be possible to obtain some information on the site of thiamine absorption in man. The case history is described below of a man who was admitted to hospital with a mesenteric thrombosis which required resection of all of his intestine except for four inches of jejunum and the distal half of the colon. Following recovery from the operation, his ability to absorb thiamine was tested at three different dose levels, 1.0 mgm., 5.0 mgm., and 20 mgm. after vitamin repletion. The results are presented in Figures 27, 28 and 29, where they are compared with results in control subjects. In Figure 27, the results found in a

FIGURE 27. The linear relationship between the reciprocal of the dose of radioactive thiamine (THCl) given orally and the reciprocal of the cummulative 72 hour urinary radioactivity in normal subjects, a malnourished alcoholic, and a patient with intestinal resection.


INTESTINAL RESECT Vmax 1-10 Km=7.1
ALCOHOLIC Vmax 1-55 Km=6.6
NORMAL Vmax 4-55 Km=8.0
Intestinal Resection
Alcoholic
Normal
RECIPROCAL 35S—THIAMINE EXCRETED (mg)
RECIPROCAL DOSE mg
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
-0.2
-0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

malnourished alcoholic are shown here for comparison although this subject will not be discussed until the next chapter.

Clinical Presentation

In 1954 at the age of 60 years, this white male patient underwent an extensive subtotal gastric resection (Billroth I) in which more than 75% of the proximal portion of the stomach was removed. In June 1966, he was admitted to hospital in mild congestive heart failure with atrial fibrillation which was subsequently controlled by digoxin and a low sodium diet. A Schilling test at that time showed 7.3% recovery. In May 1967, he was readmitted with signs of intestinal obstruction. Laparotomy revealed a mesenteric thrombosis and all of the intestine except for the proximal four inches of the jejunum and the distal half of the colon were removed, an end-to-end anastomosis being performed between the jejunum and transverse colon. He recovered from this operation but developed diarrhoea, passing five or six bulky stools daily with inability to maintain his weight. One month postoperatively, he was transferred to the metabolic unit for study. Clinical examination revealed evidence of weight loss, glossitis with lateral margin atrophy of the filliform papillae. Hyperkeratosis on his arms and legs and decreased

vibration sense at the ankles. He had controlled atrial fibrillation and his weight was 45 kilograms. He was given a 50 gram fat, 120 gram protein and 1000 mgm. sodium diet which helped to control his diarrhoea and to maintain his weight.

Two weeks later, the following investigations were done.

Haematological findings. The patient had been given 1000 ml. of whole blood during the operation. On transfer his haemoglobin was 10.9 gm. per cent, red cells were 2,810,000 per c.m.m., P.C.V. 38 per cent, white cells were 4,500 per c.m.m. Stained blood films revealed moderate hypochromia; he had a serum iron 22, total iron binding capacity 210 and a bone marrow was normoblastic but showed no free iron. His anaemia failed to respond to oral ferrous sulphate but did respond to intramuscular iron. Serum B_{12} was 89 μμgm/ml. and serum folate was 2.3 mμg/ml.

Biochemical findings. Serum Na^+ was 136 meq/l., K^+ 3.9 meq/l, Ca^{++} 8.5 meq/l. Alkaline phosphatase was 10 King-Armstrong units. Total serum proteins were 5.5 gm. (albumin 41.1%, α_1 8.9%, α_2 16.9%, β 9.7% and γ 23.4%.) Serum creatinine was 0.8 mg %, protein bound iodine 6.2 mgm. per 100 ml. and total fasting serum lipids 160 mgm. per 100 ml. The prothrombin time was initially 5 second prolonged but promptly connected with parenteral administration of

Vitamin K_1.

Hepatic and Intestinal Anatomy. A liver biopsy revealed mild fatty metamorphosis. The architectural pattern was well preserved, there was no fibrosis seen, the findings being consistent with malnutrition. The barium follow through showed a normal mucosal pattern (Figure 30) and a section taken from the proximal and of the resected intestine confirmed the presence of a normal villous pattern. (Figure 31)

Absorption Tests. Glucose absorption was abnormal; the fasting level was 67 mg% but the blood glucose failed to rise above 97 mg% following an oral dose of 50 gm. of glucose. Xylose absorption showed 0.5 gm. excreted in 5 hours following 25 gm. oral dose. The absorption of vitamin B_{12} (Schilling Test) one year prior to his intestinal resection was 7.3% but were repeated after the operation was zero without intrinsic factor following a test dose of 1 µg and 1.3% when given with 50 mgm. of hog intrinsic factor.

The results obtained indicate considerable reduction in the serum levels of radioactivity; the rate of rise in cumulative urinary excretion and in the total amount excreted in the 72 hour period at all three dose levels. (Figures 28 and 29) In Figure 27 the reciprocals of the oral doses given are plotted against the reciprocals of

FIGURE 28. Comparison of the patterns of radioactivity seen in a normal subject and a patient with an intestinal resection after 1.0 mgm. of 35S-thiamine hydrochloride (THCl) orally and 200 mgm. flushing dose.

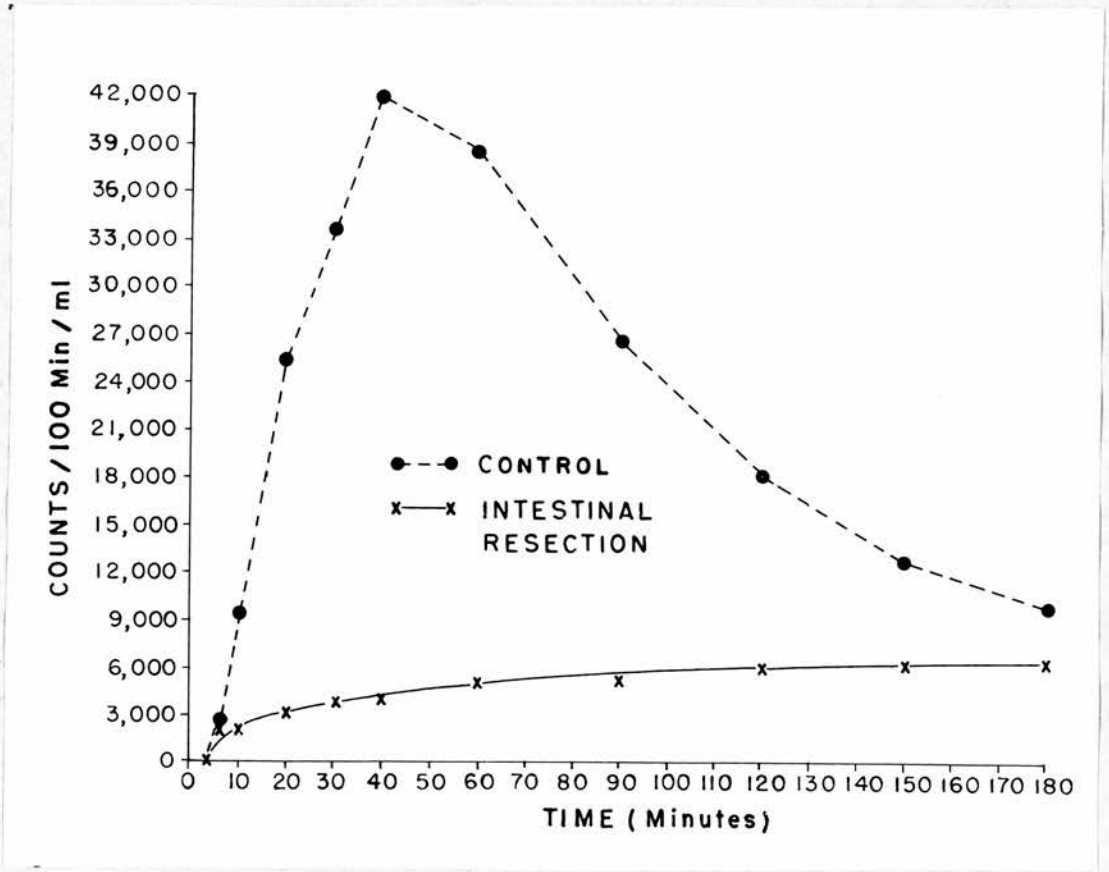

42,000
39,000
36,000
33,000
30,000
27,000
24,000
21,000
18,000
15,000
12,000
9,000
6,000
3,000
0
COUNTS / 100 Min / ml
CONTROL
INTESTINAL RESECTION
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
TIME (Minutes)

FIGURE 29. Cummulative urinary excretion of radioactivity after 1.0 mgm. oral 35S-thiamine hydrochloride (THCl) and 200 mgm. flushing dose in a normal subject and a patient with an intestinal resection.

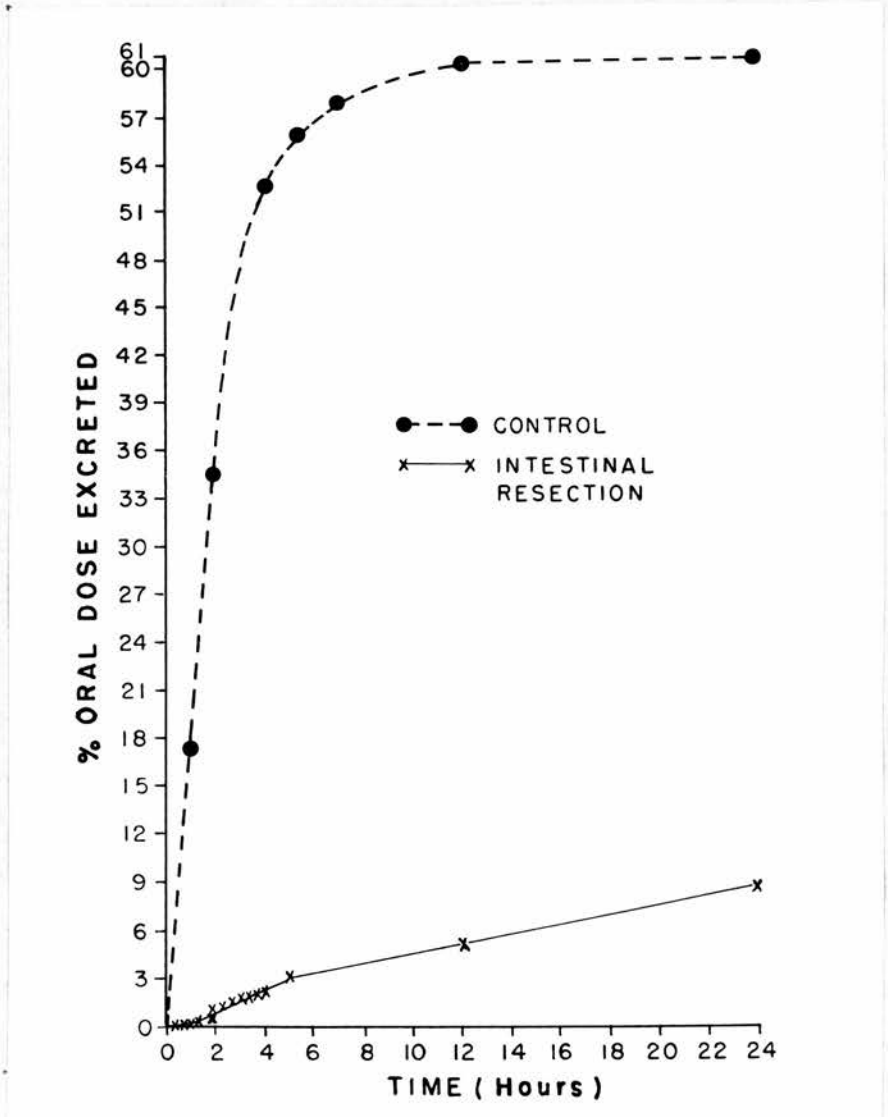

% ORAL DOSE EXCRETED
61
60
57
54
51
48
45
42
39
36
33
30
27
24
21
18
15
12
9
6
3
0
0 2 4 6 8 10 12 14 16 18 20 22 24
TIME (Hours)
CONTROL
INTESTINAL RESECTION

FIGURE 30. Barium meal and follow through examination showing the extent of the intestinal resection and the normal pattern of the remaining jejunal mucosa.

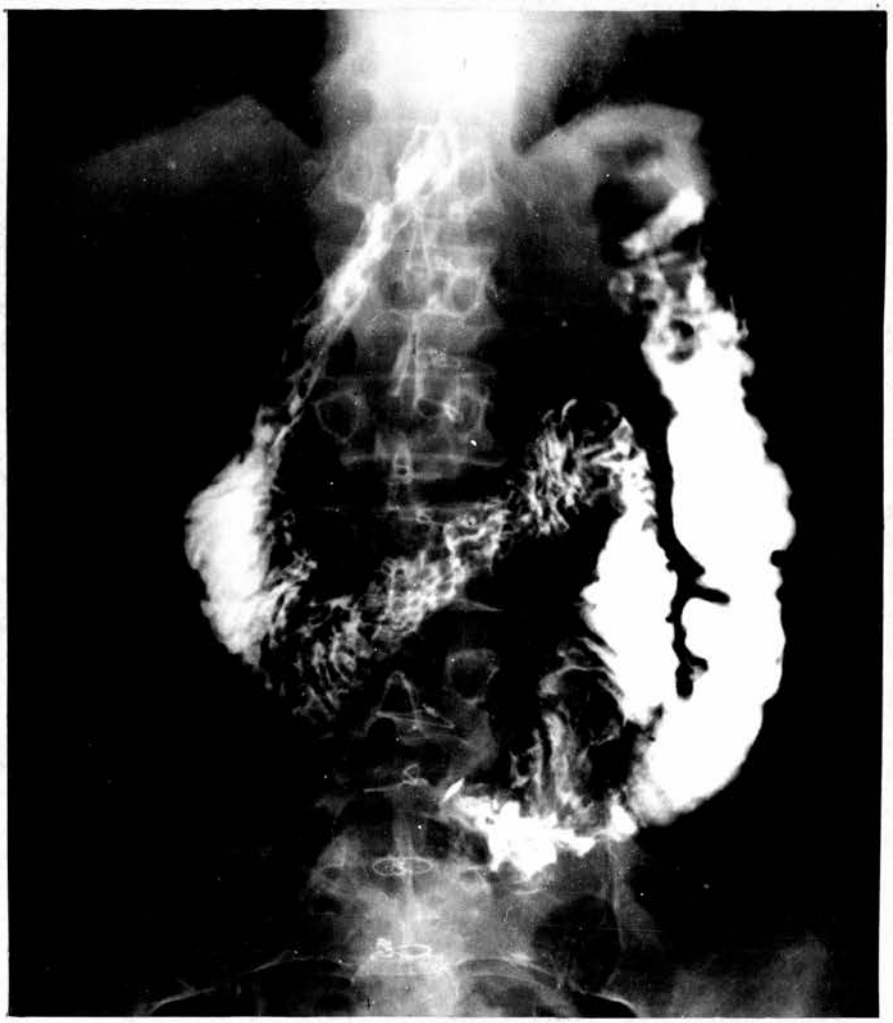


FIGURE 31. Section from jejunum of patient showing a normal villous pattern.

the total 72 hour excretion of thiamine. The results obtained in controls is shown for comparison. It will be seen that the points again fall on a straight line. The maximum amount of thiamine which can be absorbed from a single dose by this patient (V-max), 1.1 mgm. as compared with 4.5 mgm. for the controls. It is of interest that the patient excreted only 5.0% of the 20 mgm. oral dose in the 72 hours i.e. 1.0 mgm. which approximates the predicted excretion from a saturating dose. This is true also of the 20 mgm. dose given to control subjects (Figure 26).

DISCUSSION

The results agree with the findings of Melnick et al., 1945; Friedmann et al., 1948 and Morrison and Campbell, 1960, that there appears to be an upper limit to the amount of thiamine that can be absorbed from a single oral dose. It was found that on average 4.5 mgm. of thiamine was the maximum amount which appeared in the urine following any saturating dose over the range tested.

Many workers have studied thiamine absorption but the findings are difficult to correlate due to the variations introduced by the different experimental animals used, the lack of uniformity in the size of dose studied, and the different methods used to measure the amount of absorption

that has occurred. Melnick et al., (1945) stated that in normal subjects receiving nutritionally adequate diets, the urinary excretion of water-soluable vitamins, or their derivatives, was directly proportional to the quantity consumed. The upper limits for thiamine tested by Melnick et al. was 7.5 mgm. and inspection of their data reveals that a break in the linear dose-response relationship occurred at an intake of about 5.0 mgm. per day. Friedmann et al., 1948, concluded that the intestinal absorption of thiamine and hence its excretion, was extremely limited. The maximum amount which could be taken orally without a resultant increase of faecal thiamine was about 5.0 mgm. per day. Previously, Schultz et al. (1938) had reported that, in subjects receiving 5.0 mgm. of thiamine daily, almost all of an additional dose of 5.0 mgm. thiamine was recovered in the faeces.

Morrison and Campbell (1960) investigated the absorption of thiamine in six normal male subjects. The subjects were given of 1.0 mgm. to 20 mgm. of thiamine after breakfast. The excretion of thiamine was measured in divided urine samples by the thiochrome method until it returned to control levels. The results are shown in Table 31. It was found that the excretion of thiamine, expressed as a percentage of the oral dose, decreased markedly with doses greater than 2.5 mgm. Increasing the dose from 2.5 mgm.

Table 31. Effect of size of dose on urinary excretion of thiamine

Dose	Thiamine, mean net excretion	
mgm	mgm	% of dose
1	0.276	27.6 ± 4.3*
2.5	0.525	21.0 ± 3.6
5.0	0.435	8.7 ± 0.7
10	0.640	6.4 ± 0.6
20	0.740	3.7 ± 1.1

*Standard error of the mean.

to 20 mgm. increased the amount of thiamine excreted by only 0.2 mgm. The results obtained by Japanese workers who measured the 24hr. urinary excretion of thiamine following oral doses of 2,6,10, 25, 50 and 100 mgm. of thiamine daily are summarised in Table 32. It was concluded that the pattern of intestinal absorption conforms to the view that an upper limit exists to the amount of thiamine absorbed from a single oral dose.

Watanabe, 1951, showed that doses of thiamine up to 400 µgm. were readily absorbed by the rat based on urinary output. Middleton and Grice, 1964, gave 20 µgm. of 35S-thiamine by stomach tube to 200-250 gm. Wistar rats and analysed the radioactivity remaining in the intestine at varying times after the oral dose. It was found that 88% of the dose had been absorbed after six hours and that 20% was retained in the liver. In a second series of experiments, the same oral dose was given and the amount of radioactivity excreted in the urine and faeces in the subsequent 48 hours was measured. It was found that approximately 34.3% of the dose was excreted in the urine and 10.8% in the faeces - presumably 42.9% being retained in the body. These findings are in good agreement with earlier findings of Middleton and Morrison, 1962, who found that 29.1% $\pm$ 5.8% of a 20 µgm. dose was excreted in the urine in a four day period with 5.5 $\pm$ 3.3% in the faeces.

Table 32. Relationship between the amount of the oral administration and the urinary excretion in the case of ordinary thiamine

Amount Administered (mgm.)	Time after Administration					
	I	II	III	IV	V	Ratio of V to the Amount Administered (%)
	Total Amount on Previous day (µgm.)	3 hrs. (µgm.)	4–24 hrs. (µgm.)	Total Amount (µgm.)	Difference between I and IV (µgm.)	
2	348	240	387	627	279	13.9
6	373	282	525	807	434	7.2
10	398	340	583	923	525	5.2
20	424	733	689	1422	998	4.9
50	434	953	1229	2183	1749	3.4
100	419	1343	1892	3238	2816	2.8

Da Silva and Ivy, 1961, gave 40 mgm. of thiamine orally for six days to 20 kgm. dogs and measured the daily urinary and faecal excretion of thiamine by the fluormetric method of Burch et al., 1952. The average daily feacal excretion of thiamine in excess of that in the control period was approximately 46% of the dose with 16% appearing in the urine. Presumably, 38% was either retained in the body, destroyed or present as undetected metabolites in urine or faeces. This suggests that approximately 21.7 mgm. of thiamine were absorbed daily. These workers also found that approximately 30% of a 10 mgm. of thiamine was absorbed from the Thiry fistula of the jejunum or ileum in one hour, no significant difference being found between the two portions of the intestine. It was possible to recover all of the thiamine introduced into the loop and there was no evidence of thiamine destruction when incubated invitro with fluid obtained from washing the loop. Therefore, although workers had varied in their choice of animal, in their choice of size and number of different dose levels investigated and in their methodology, it was apparent that there was an upper limit to the amount of thiamine that could be absorbed from a single dose and in man this was between 8-14 mgm. The intestinal capacity to absorb the vitamin had not been studied in the rat but increased faecal excretion occurred in a 100 gm. rat when

0.12 mgm. were fed. On a body weight basis, therefore, maximum absorption would be greater than 1.2 mgm./kgm. for the rat, approximately 1.1 mgm./kgm. for the dog and 0.07 mgm./kgm. for man. This suggested that a species difference existed and that the intestinal capacity to absorb thiamine, on a body-weight basis, was greater in the rat than in the dog, and greater in the dog than in man.

It is of interest that plotting the reciprocals of the means for the different groups provides a relationship between, the dose given and the total oral thiamine excretion, which can be represented by an equation of the Michaelis & Menten type (1913). It may be that in the fasting state, the oral dose given is directly related to the initial concentration in the lumen of the intestine and that the total excretion, under test conditions, represents the average rate of absorption. Conformity to Michaelis-Menton kinetics, however, does not necessarily indicate that an enzymic process, with a rate-limiting step, is involved. As stated by Fisher and Parsons, 1953, if absorption on to some carrier complex were the rate limiting process, the same kinetics would obtain. But, it does show that the results are not explainable on the basis of simple diffusion alone.

Since the measurement of thiamine absorption is

indirect, it is possible that the rate limiting factor may not be in the intestinal mucosa but in the transport system of the blood, in the tissues or imposed by renal excretion. Thiamine may be transported in the blood in the phosphorylated form coupled to a protein carrier (Baker et al., 1967) and it is possible that this mechanism could become saturated. However, the total body store of thiamine in man has been estimated to be 30 mgm. and repeated flushing doses of 100 mgm. each have not altered the pattern of excretion or the total amount of radioactivity excreted. For the same reasons, it is unlikely that tissue binding or utilization could introduce a rate limiting step especially as 90% of the radioactivity, during the period of maximum absorption, is excreted in the form of thiamine. At high blood levels, thiamine is actively secreted by the kidney tubules (Haugen, 1961). Excretion of intravenous thiamine is maximal during the first half hour whereas the peak serum level of thiamine and the maximal period of excretion of the oral dose is at about 90 minutes by which time the rate of excretion is far below its initial levels. At no time during the period of absorption of the oral dose is there evidence that the excretory mechanism is being saturated. An estimate of the amount of thiamine absorbed has been made by measuring the total excretion of radioactivity during the 72 hour period following the

oral dose but, most of the activity is excreted in the first 12 hours.

It is possible to show that absorption of thiamine from the intestine occurs during the period when the concentration of thiamine in the portal vein, due to the flushing dose, is greater than the concentration in the intestine. However, the form in which a large intravenous dose would be transported has not been determined and it is not known whether this could represent evidence of passage of thiamine against a concentration gradient. Consequently, the most likely explanation for the upper limit to thiamine absorption would seem to be that thiamine is absorbed by active transport, fascilitated diffusion, or a non-active process involving a rate limiting step, for example, absorption on to a cell surface.

The mechanism of absorption was investigated by Polin et al., 1963a, in White Leghorn hens. A duodenal loop 1-13 cm. long was prepared so as to exclude the bile ducts and the pancreatic tissue. Following an injection of 2.0 or 4.1 μmoles of thiamine into the loop, the incision was closed and the animal returned to the cage for 30 minutes before sacrifice. The loop was then removed, and both the intestine and its contents homogenized. Following extraction, an aliquot of the supernantant was analysed for thiamine. The experiments were also repeated

in the presence of 28 μmoles of amprolium. The relationship between the amount of thiamine absorbed and the dose given conformed to the Michaelis-Menton relationship graphically represented by the Lineweaver-Burk plot. (Lineweaver and Burk, 1934). The results in the presence of amprolium suggested that competition for absorption existed between amprolium and thiamine. The authors concluded that amprolium and thiamine are absorbed from the duodenum by an active carrier mechanism rather than by simple diffusion. Further work by the same authors (Polin et al., 1963b), confirmed their earlier findings and excluded the possibility that a chemical complex of thiamine and amprolium within the intestine could account for the interference effect. A further publication by the same authors three months later, however, showed that thiamine absorption from the ligated duodenal loop in 3-5 week old chicks produced a dose absorption curve which was linear passing through the origin. (Polin et al., 1964). From 250 μgm. to 50 mgm. in one ml. volume were introduced into the loop and the relationship between the amount absorbed and the amount injected into the loop was described by the equation $y = 0.09 + 0.73x$ mgm. until the dose exceeded 10 mgm. Higher doses produced a curving phase interpreted as representing thiamine toxicity or osmotic imbalance.

Turner and Hughes, 1962, investigated the absorption

of thiamine using the everted sac technique of Wilson and Wiseman, 1954, and the loop technique of Fisher and Parsons, 1949, in both rats and hamsters. Thiamine was estimated by the fluorimetric method of Bessey et al., 1949. Varying concentrations from 20 μmole/l. to 60 mole/l. were added to the mucosal side in the first experiments, and the course of vitamin absorption was determined by measuring the initial and final concentrations of vitamins in the mucosal solution but little change in the concentration was detected. Similarly, when the initial concentration on the mucosal side was compared to the accumulation on the serosal side, it was found that the final concentration of thiamine on the serosal side was always lower than the mucosal concentration and the system never reached equilibrium. Consequently, passage against a concentration gradient was not demonstrated. In experiments in which the initial concentration was the same on both the mucosal and serosal sides (20μmoles/l.), there was a loss of thiamine from the serosal side and an increase in the vitamin content of the tissue. No movement of thiamine from seroasal to mucosal side was detected but jejunal sacs incubated without added vitamins showed loss of thiamine from the tissue preferentially to the mucosal side $36 \pm 5.3 \times 10^{-11}$ moles/cm./hour.

The effect of cytotoxic inhibitors such as cyanide was

to increase the amount of vitamin passing across the mucosa. The authors concluded that in the absence of evidence of movement against a concentration gradient and failure to demonstrate reduced absorption in the presence of inhibitors, it was thought that thiamine absorption obeyed Fick's Law of diffusion (Hober, 1945). One complicating factor was to evaluate the influence of water movements. The concentration differences resulting from the absorption of solute could be masked by movements of water. Also, a linear relationship between the rate and concentration gradient, while consistent with diffusion, does not exclude absorption by enzymic mechanisms. A similar result could be obtained in a transport process mediated by enzymes provided the substrate concentration is well below the Km. It is also possible that the authors used too high a dose and were already dealing with a saturated system in which comparatively small changes in concentration were not detected. Other problems in interpretation result because of doubt about the biological integrity of the preparations at the end of the incubation period. They were shown to respire actively for the whole period and to be able to oxidise glucose but histological sections were described as "although showing no gross damage, nevertheless, were not in as good condition as those reproduced by Fisher and Parsons, 1949." There were no tests to

demonstrate inhibition of the passage of thiosulphate nor is it certain that all of the thiamine was in a form measured by the assay method.

Spencer and Bow, 1964, also investigated thiamine absorption in hamster intestine using 35S-thiamine hydrochloride. Their report contained no evidence of viability or evidence that the preparation was not damaged and water movements were not investigated. Their conclusions agreed with the findings of Turner and Hughes, 1962, for they were unable to demonstrate movement against a concentration gradient and suggested the passage of thiamine occurred from serosal to mucosal side.

Other workers also failed to find any evidence for active absorption of thiamine in the rat invivo, at least when relatively high doses of the vitamin were used. (Stockholm et al., 1941). Watanabe, 1951, has reported that young rats absorb doses of 120, 240, or 400 µgm. of unlabelled thiamine with about the same efficiency, indicating that there is no threshold value within this range. Draper, 1958, showed no impairment of radiothiamine absorption in weanling rats fed on synthetic thiamine deficient basal diet supplemented with 50 µgm./gm. of thiamine hydrochloride although the experiments were carried out after a 24 hour fast and the animals were under light ether anaesthesia. These findings are in contrast, however, to those

of Magyar and Gabor, 1949, who reported that absorption of thiamine from an isolated loop of intestine was reduced by prior administration of large doses of thiamine or other B-vitamins; to those of Ventura et al., 1963, who showed comparatively greater absorption of small than of high doses of thiamine, and to Polin et al., 1963a-b; who employing intestinal loops of chicks, suggested that thiamine uptake may be an active process superimposed on some passive absorption. Active transport of thiamine has been shown to occur in other tissues of the body. Sharma and Quastel, 1965, using rat brain cortex slices in Krebs-Ringer phosphate medium containing glucose and labelled thiamine at an initial concentration of 0.2 μmole, 0.6 μmole and 1.0 μmole, concluded that thiamine transfer into the brain cell was by a carrier mediated mechanism depending upon the operation of the sodium pump. They also found, however, that at high external concentrations of thiamine, simple diffusion of thiamine into the tissues caused the concentration ratio of total labelled thiamine (tissue:medium) to become approximately unity. It has also been shown that the transport of thiamine into the bacterium, Lactobacillus fermenti, is dependent upon an external source of energy and that this can be utilized in the form of ATP (Neujahr, 1963).

Ventura and Rindi, 1965, noted that with several

substrates, for example, basic amino acids (Hagihira et al., 1961), pyrimidines (Schanker and Tocco, 1960), d-xylose (Csaky and Lassen, 1964), the demonstration of an uphill intestinal transport could be achieved only using very low initial concentrations and applied this principle to the study of thiamine transport in vitro. Everted sacs (8 cm. long) from the upper small intestine of rats (Wister strain 100-120 gm. body weight), prepared according to Wiseman, 1961, were incubated with an initial concentration of 0.21 µmole/l. on both sides of the sac. Thiamine was determined by a micro modification of the thiochrome method. (Ass. Vitamin Chemists, 1951). A net transport of thiamine against a concentration gradient was demonstrated, the serosal concentration of the vitamin increasing up to 2.1 times the initial one. The effects of metabolic inhibitors and decreased incubation temperature depressed the serosal accumulation of thiamine supporting the concept of an active transport mechanism. The structural thiamine analogue, pyrithiamine, significantly inhibited the transport of thiamine against a concentration gradient. Since pyrithiamine is a potent inhibitor of the thiamine phosphorylase from the rat intestine (Cerecedo et al., 1954), it was suggested that phosphorylation might be the basic mechanism of the intestinal uphill transport of thiamine. It is interesting to note

that in several experiments in which the initial concentration of thiamine was 21 or 2.1 μmole/l., no evidence of an uphill transport was found, thus confirming the results of Turner and Hughes, 1962. These results should probably be accepted with reservations since when working with an initial concentrations of 0.21 μmole./l., non-specific binding to tissues has been shown to occur, but the authors have demonstrated a concentration gradient. One wonders about the accuracy of the thiochrome method in this range of concentration and the unexplained reason why the serosal surface alone was rinsed with 0.01 N hydrochloric acid.

The possibility that thiamine is phosphorylated during its intestinal absorption has been suggested by some authors (Linneweh and Muller, 1940) although denied by others (Stockholm et al., 1941). Several experiments invitro (Tauber, 1938; Cerecedo et al, 1954) have demonstrated that intestinal tissue is able to phosphorylate thiamine. However, the relationship between thiamine phosphorylation and intestinal absorption has never been clearly defined although Machida, 1955, found thiamine phosphates in the wall of the isolated intestinal tract of the rat after incubation with thiamine. This question was re-examined by Rindi et al., 1966. They studied the absorption of thiamine hydrochloride and thiamine-propyl

disulphide (TPS), a thiamine derivative rapidly absorbed and transformed into thiamine by the intestinal mucosa. (Ventura et al., 1963b; Takenouchi and Aso, 1963) using a modification of the Cori technique (Ventura et al., 1963a; Ventura et al., 1963b) on the small intestine insitu. The rats were sacrificed two hours after introduction of the compound into the intestine, the thiamine being determined by the thiochrome method (Ass. Vitamin Chemists, 1951) with modification by Mickelsen et al., 1945, and the thiamine-propyl disulphide (TDS) being determined by the method of Ventura et al., 1966. These authors found that a significant increase in phosphorylated thiamine (Thiamine disulphide) occurred in the intestinal wall two hours after the introduction of equivalent amounts of thiamine hydrochloride or TPS.

The experiments of Rindi and Ventura, 1966, have not established the relationship between thiamine phosphorylation and thiamine absorption. The accumulation of thiamine phosphate during absorption cannot be taken as evidence for its participation in transport as many cells increase the concentration of intermediates of metabolism upon the addition of utilizable thiamine. In 1933, Wilbrandt and Last, proposed that sugars were phosphorylated at the cell membrane as part of a transport mechanism. Many workers found accumulation of sugar phosphates

in the intestinal epithelium during sugar absorption (Lundsgaard, 1933; Laszt and Sullmann, 1935; Nagasawa, 1957; Naito, 1914 and Papadopoulos and Roe, 1957).

This evidence was supported by the inhibition of glucose absorption by iodoacetate and phlorizin, believed to be a specific inhibitor of phosphorylation reactions. However, as the years passed, evidence accumulated that cast doubt on the validity of the theory. Some of the most convincing evidence against the theory came from accumulated data on the specificity of sugar transport (Wilson, 1962), and the work of Landau and Wilson, 1959. In these studies, radioactive galactose was used to label the glucose-6-phosphate pool within the tissue and the proportion of transported glucose passing through the pool was estimated by the radioactivity of the transported glucose recovered on the serosal side of the intestinal segments. These experiments indicated that less than 10% of the transported glucose could have passed through the pool. More recently, Crane et al., 1961, have suggested a direct coupling of the sodium transport system to that for sugars.

Gassmann and Sandner, 1967, presented evidence that phosphroylation of thiamine occurred in the lumen of the intestine and that extracts of pancreatic tissue were able to phosphorylate thiamine invitro. Ligation

of the pancreatic duct was associated with a decrease in thiamine absorption. The explanation of the results were open to other interpretations. Phosphorylation of substances in the intestine occur near to the brush boarder so that the constant traumar and shedding of cells into the lumen could explain the phosphorylated thiamine in the intestinal tract. The demonstration of the ability of pancreatic tissue to phosphorylate thiamine does not necessarily mean that it plays an important role and ligation of the pancreatic duct must alter lymphatic drainage and the nature of the intestinal contents. The experiments might have been more convincing had the pancreatic juice been given orally together with thiamine after duct ligation to show that the absorption defect was corrected.

The mechanism of thiamine absorption has been investigated by many workers using a variety of techniques in different animal species. The results obtained have often been inconclusive and at times contradictory. There is no agreement on whether active transport or passive diffusion is involved in the absorption of thiamine from the intestine and what part, if any, phosphorylation plays in the process. It is not known whether significant breakdown of thiamine occurs in the intestine or what part re-excretion into intestine plays at blood levels obtained

following normal dietary intake.

Comment

The results presented in this chapter suggest that in man thiamine hydrochloride absorption is rate limited. As shown previously there is little evidence of significant entero-hepatic circulation, breakdown of thiamine or re-excretion into the intestine. The requirement for a special mechanism of absorption, perhaps requiring receptor sites, makes the system vulnerable in the presence of malnutrition or states interfering with the production of substances concerned with the transport process.

CHAPTER VI

PATTERNS OF 35S-THIAMINE HYDROCHLORIDE ABSORPTION IN THE MALNOURISHED ALCOHOLIC

Introduction

If a specialized mechanism was responsible for thiamine hydrochloride absorption, then it seemed possible that malnutrition or reduced synthesis of proteins secondary to liver injury might be associated with thiamine malabsorption. This possibility was further supported by the known high incidence of thiamine deficiency among alcoholic subjects and consequently I decided to investigate the possibility further.

Thiamine deficiency syndromes in the malnourished alcoholic have been attributed to an inadequate intake (Leevy, 1967) or defective utilization of this vitamin (Fennelly et al., 1967; Leevy and Baker, 1968). It has been assumed that malabsorption may also produce thiamine depletion because of occasional inability to achieve normal circulating levels of this vitamin by oral therapy (Leevy et al., 1965). As shown in Chapter II, patients with idiopathic steatorrhea characteristically exhibit decreased absorption of 35S-thiamine which returns to normal following treatment with a gluten-free diet. My

preliminary work suggested that alcoholics often exhibit a market reduction in intestinal absorption of radioisotopic thiamine hydrochloride, attributable to ethanol toxicity, nutritional deficiency, or both (Thomson et al., 1968). The influence of malnutrition was subsequently confirmed by Tomasulo et al., 1968, using the test which I have described here and published (Thomson, 1966). The present studies were undertaken to (a) determine the effect of ethanol and nutritional deficiency on absorption kinetics; and (b) evaluate the influence of various therapeutic regimens on thiamine absorption in malnourished alcoholics.

Methods and Subjects Studies

Twelve alcoholics without malnutrition or evidence of hepatic disease, and 17 malnourished alcoholics with biopsy evidence of fatty liver or cirrhosis were selected for study. None of the patients had clinical evidence of intestinal, cardiac, or renal disease. Hepatic status was evaluated in alcoholic patients by the serum bilirubin (Molloy et al., 1937) serum glutamic pyruvic transaminase (Wroblewski and La Due, 1956) indocyanine green (ICG) clearance (Leevy et al, 1967), and liver biopsy before and after treatment. Estimated hetapic blood flow was determined by the method of Leevy et al., 1962. Intestinal

function was investigated by stool examination, upper gastrointestinal x-rays, and jejunal biopsy in selected patients. Nutritional status was evaluated by studies of circulating levels of vitamins, serum protein electrophoresis, serum cholesterol (Bloor and Knudson, 1966), and serum magnesium (Hansen and Freier, 1967). Vitamins A_1 and E were measured chemically. Thiamine, riboflavin, nicotinic acid, folic acid, vitamin B_{12}, vitamin B_6, biotin, and pantothenic acid were assayed by protozoologic techniques (Baker and Frank, 1969).

The 35S-thiamine, specific activity 356 mc/gm. was radiochemically pure when tested in the following chromatographic system: N-propanol/water/IM acetate buffer pH 5.0 (70:20:10) and pyridine/acetic acid/water (20:2:80). Labelled thiamine was diluted with non-radioactive thiamine hydrochloride so that each test dose contained from 1.0 mgm. or 5.0 mgm. of thiamine hydrochloride and 10 μc of radioactivity dissolved in 20 ml of water. After an overnight fast, a parenteral injection of 200 mgm. of non-radioactive thiamine hydrochloride was given immediately, after which the radioactive material was given orally. Arterial blood was collected via an indwelling catheter at 0, 3, 6, 10, 20, 30, 40, 60, 90, 120, 150, and 180 minutes. Urine was collected hourly for the first 5 hours and then after 12, 24, 48, and 72 hours. Blood and urine

radioactivity was determined in a Packard Tricarb scintillation counter as previously described.

Ethanol administration

Twelve healthy subjects were given ethanol, either orally or intravenously, to investigate the influence of ethanol on the absorption of 35S-thiamine hydrochloride. Thiamine absorption patterns were compared with and without ethanol in the blood and intestines by tests conducted one week apart with their order randomized. Studies were obtained under conditions of hepatic vein catheterization in 2 patients to see if ethanol altered the route of absorption; in these instances ethanol produced no significant increase in estimated hepatic blood flow. Nine subjects were given 180 ml of 79.39 g/100 ml of alcohol diluted 1:3 with water and administered in 6 doses at 2 hour intervals after an overnight fast. Three subjects received intravenous ethanol (1.5 gm/kgm body weight in a 20% solution) infused at a constant rate for 1 hour after an overnight fast. Blood was obtained at hourly intervals during and immediately following the administration of ethanol for measurement of serum ethanol and lactate. Five mgm of 35S-thiamine hydrochloride together with a 200 mgm. non-radioactive flushing dose of thiamine hydrochloride was given by tube at the time of the last dose of oral ethanol or immediately after the ethanol infusion was stopped.

Absorption in malnourished alcoholics

Thiamine hydrochloride absorption was studied in 12 alcoholic patients with biopsy evidence of fatty liver without clinical or laboratory evidence of steatorrhea. Each patient had clinical stigmata attributed to vitamin deficiency including glossitis in 8 and peripheral neuropathy in 7. Serum protein electrophoreses were abnormal in each of the patients. A significant reduction in circulating levels of folic acid was present in 8, vitamin E in 7, vitamin B_6 in 5, thiamine in 4, vitamin B_{12} and pantothenic acid in 2, and biotin in 1 patient. Blood sugar, BUN, hematocrit, and serum magnesium were normal. Intestinal biopsies and gastrointestinal series were normal. Each of the patients had abnormal ICG clearance, 85% an increase in serum glutamic pyruvic transaminase, and 50% an elevated serum bilirubin.

Absorption studies were conducted within 24 hours of admission and repeated following the administration of 100 mgm. or thiamine hydrochloride and therapeutic quantities of vitamin C and the B-complex administered parenterally for 3 to 6 days. Absorption studies, nutritional evaluation, and liver biopsies were repeated following a 6 to 8 week period of a high carbohydrate (370 gm), high protein (120 gm.) diet, vitamin supplements (thiamine hydrochloride 10 mgm., riboflavin 10 mgm., niacinamind 80 mgm., pyridoxine hydrochloride 20 mgm., D-panthenol 20 mgm., d-biotin

0.2 mgm., ascorbic acid 100 mgm., folic acid 5.0 mgm., and vitamin B_{12} 100 ugm. intramuscularly daily), and a 10 day course of Oxandrolone (120 mgm. daily) to facilitate mobilization of liver fat. Three patients in this group had absorption studies utilizing hepatic vein catheterization before and after treatment.

RESULTS

Effects of ethanol

A 70% reduction in expected serum radioactivity, and a 52% decrease in expected urinary radioactivity (Figure 32) was noted in 3 of 9 subjects in whom ethanol was given orally, and 1 of 3 in whom it was given parenterally. Prior to the receipt of the ethanol, the mean serum radioactivity at 1 1/2 hours after administration of 35S-thiamine was 10,567 $\pm$ 1,230 c/100 min/ml; after ethanol it was 3,927 $\pm$ 1,014 c/100 min/ml. The mean 72 hour urinary thiamine excretion of these patients was 56.5 $\pm$ 5.9% of the administered dose; after ethanol administration, the mean urinary excretion was 27.2 $\pm$ 5.1%. One subject retested after a 4 month period showed similar reduction in 35S-thiamine absorption following the administration of ethanol. Hepatic vein catheterization showed that the route of thiamine absorption remained the same with reduced uptake of the vitamin. (Figure 33).

FIGURE 32. The radioactivity in the serum and urine after administration of 5.0 mgm. of radioactive thiamine (THCl) orally to 3 normal subjects with and without prior administration of ethanol (1.5 gm per kilo); 200 mgm. of non-radioactive thiamine was given intravenously along with the oral dose.

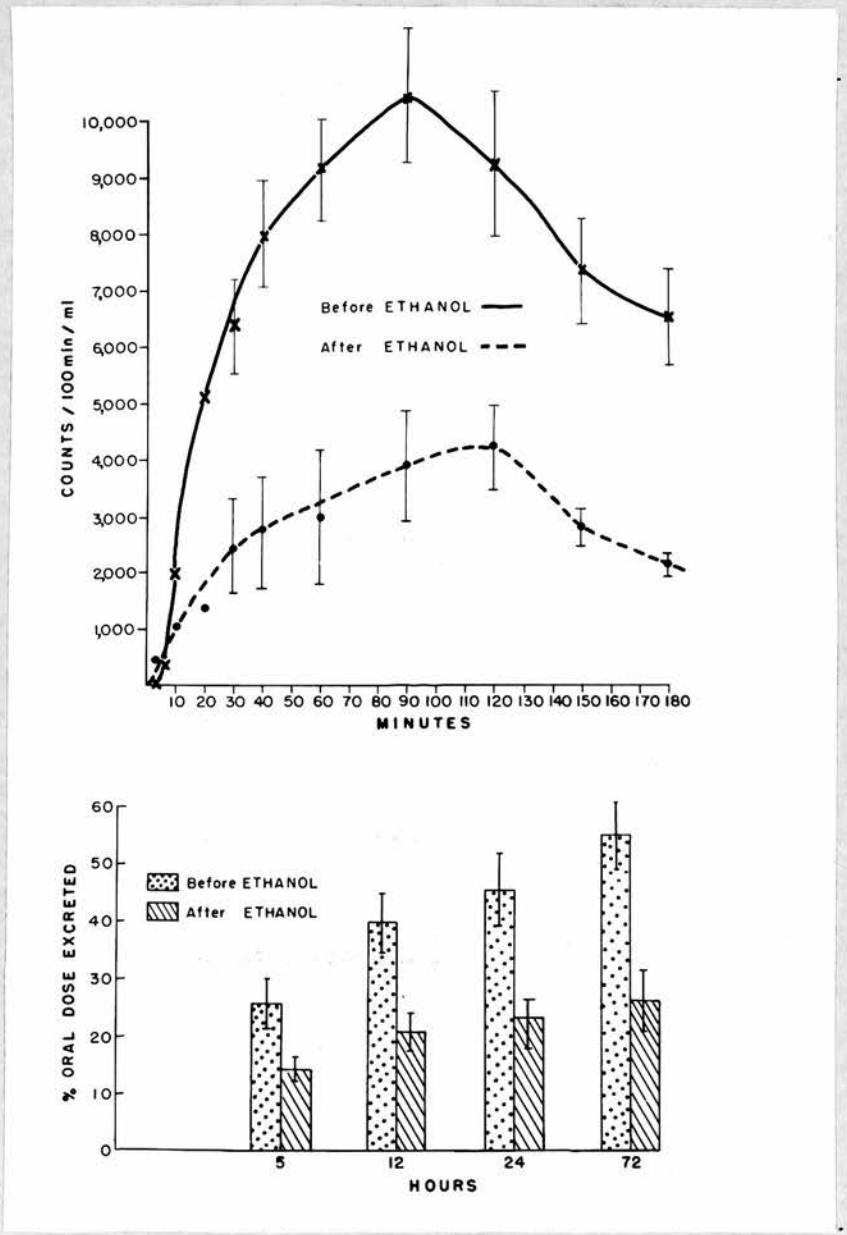
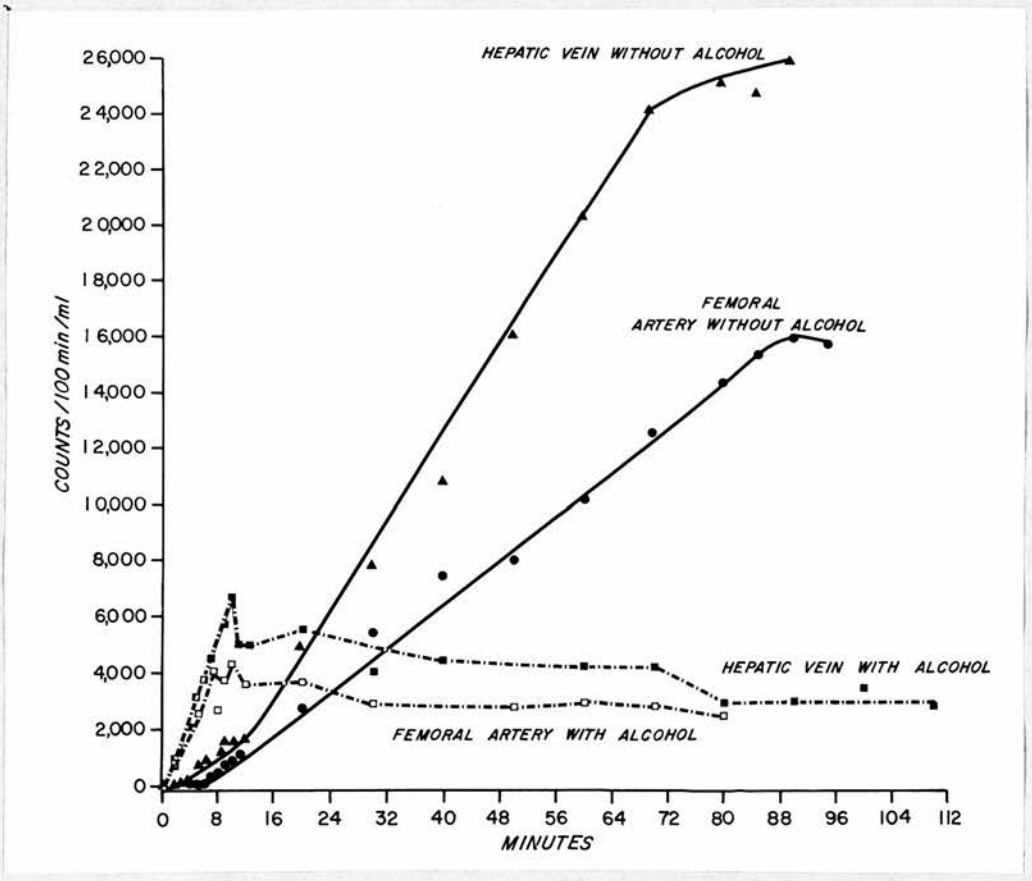

10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
COUNTS / 100 min / ml
Before ETHANOL
After ETHANOL
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
MINUTES
60
50
40
30
20
10
0
% ORAL DOSE EXCRETED
Before ETHANOL
After ETHANOL
5
12
24
72
HOURS

FIGURE 33. The radioactivity in the hepatic vein and femoral artery after administration of 5.0 mgm. of radioactive thiamine (THCl) orally to a normal subject with and without prior administration of ethanol (1.5 gm per kilo); 200 mgm. of non-radioactive thiamine was given intravenously along with the radioactive thiamine.


HEPATIC VEIN WITHOUT ALCOHOL
FEMORAL
ARTERY WITHOUT ALCOHOL
HEPATIC VEIN WITH ALCOHOL
FEMORAL ARTERY WITH ALCOHOL
COUNTS / 100 min / ml
26,000
24,000
22,000
20,000
18,000
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
0 8 16 24 32 40 48 56 64 72 80 88 96 104 112
MINUTES

Malnourished alcoholic subjects

Serum levels of radioactivity following oral administration of 35S-thiamine in alcoholics with fatty liver or cirrhosis were from 30 to 98% less than the lower level established for normal subjects. Portal and hepatic venous levels were reduced to the same extent. Pooled 72 hour urine specimens contained only 13.9 ± 3.03% of the orally administered radioactivity compared with 35.3 ± 2.16% in control subjects (Table 33). Serum and urinary radioactivity was not altered by parenteral administration of thiamine hydrochloride, therapeutic quantities of C and B-complex vitamins, or a 10 day course of Oxandrolone. Repeat studies following a 6 to 8 week period of a nutritious diet and vitamin supplements revealed increase of serum and urinary radioactivity to those of controls (Figure 34). Combined umbilical and hepatic vein catheterization demonstrated a block of absorption at the intestinal level which was corrected with prolonged receipt of a nutritious-vitamin supplemented diet (Figure 35).

DISCUSSION

The demonstration that thiamine absorption obeys Michaelis-Menten kinetics makes it possible to ascertain the mechanism whereby various factors interfere with its intestinal transport. As shown previously, a marked decrease in Vmax occurred in the patient with intestinal

Table 33a. Cummulative Urinary Radioactivity in control subjects and malnourished alcoholics

	Cummulative Urinary Excretion*			
Subjects	5 hr.	12 hr.	24 hr.	72 hr.
Control				
Mean	20.0	30.3	34.3	37.7
S.E.	1.9	3.6	3.8	3.9
Malnourished alcoholic				
Mean	7.8	10.8	12.7	13.9
S.E.	1.9	2.4	2.8	3.0
P	< 0.001	< 0.005	<0.001	<0.001

*Per cent oral dose.

S.E. = standard error.

Table 33b. Serum radioactivity in control subjects and malnourished alcoholics

	Serum Radioactivity*										
Subjects	3 min.	6 min.	10 min.	20 min.	30 min.	40 min.	60 min.	90 min.	120 min.	150 min.	180 min.
Control											
Mean	100	243	980	2,392	3,357	4,726	5,846	6,866	6,679	5,976	5,507
S.E.			358	709	808	993	924	956	776	488	43
Malnourished alcoholic											
Mean	24	189	646	1,868	2,723	3,315	3,342	3,432	3,309	1,898	1,562
S.E.			209	514	777	927	882	810	759	633	521
P							>0.05	<0.01	<0.01	<0.01	<0.01

*Counts per 100 min. per ml.

S.E. = Standard error.

FIGURE 34. The radioactivity in the serum and urine after administration of 5.0 mgm. of radioactive thiamine orally to malnourished alcoholic subjects before and after treatment; 200 mgm. of non-radioactive thiamine was given intravenously along with the radioactive thiamine.

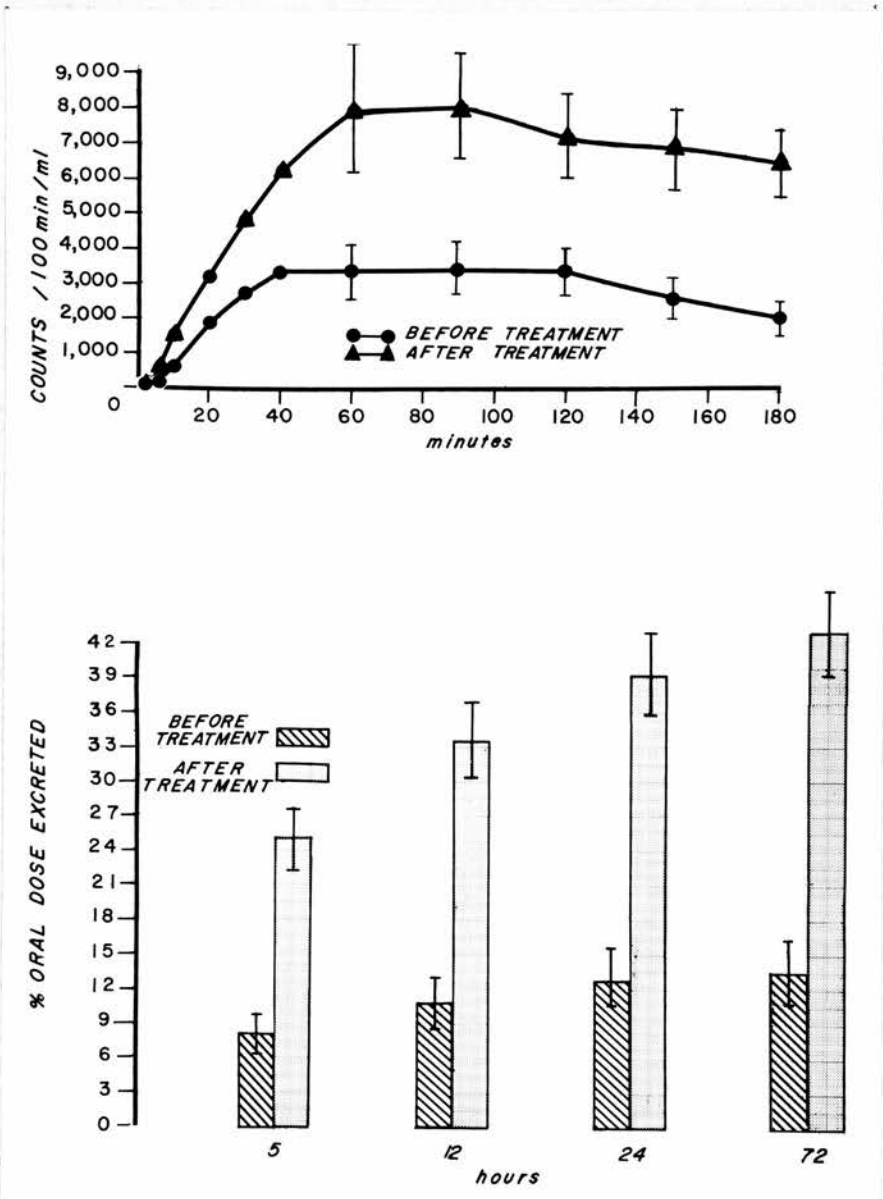
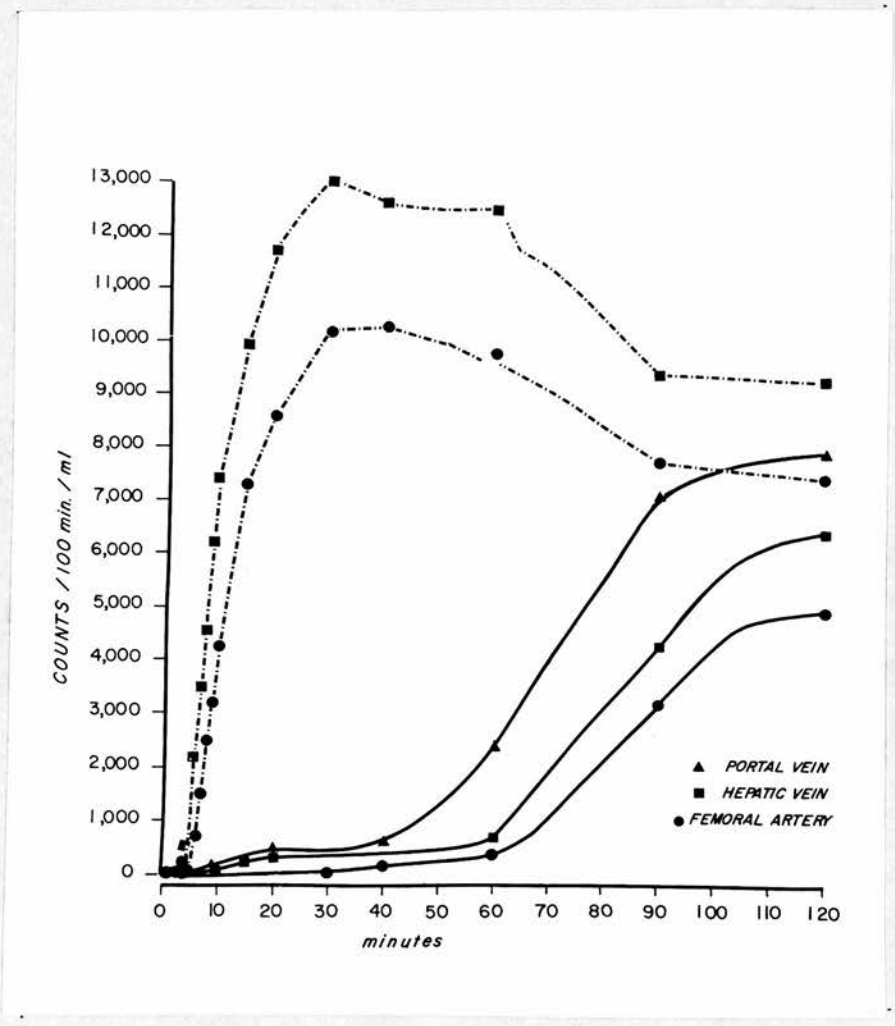

COUNTS / 100 min / ml
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
20
40
60
80
100
120
140
160
180
minutes
BEFORE TREATMENT
AFTER TREATMENT
% ORAL DOSE EXCRETED
42
39
36
33
30
27
24
21
18
15
12
9
6
3
0
BEFORE TREATMENT
AFTER TREATMENT
5
12
24
72
hours

FIGURE 35. The radioactivity in the portal vein, hepatic vein, and femoral artery before treatment in a malnourished alcoholic following 5.0 mgm. of radioactive thiamine orally. Hepatic vein and femoral artery in the same patient following treatment.


13,000
12,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
COUNTS / 100 min. / ml
0
10
20
30
40
50
60
70
80
90
100
110
120
minutes
PORTAL VEIN
HEPATIC VEIN
FEMORAL ARTERY

resection, attributed to a reduction in the total number of receptor sites. The identical reduction in Vmax noted in malnourished alcoholics is interpreted as evidence that receptor sites are damaged by prolonged receipt of ethanol, nutritional deficiency, or a combination of these factors. Time lapses required for restoration of normal thiamine absorption are attributable to need for repair or regeneration of mucosal cells with adequate receptor sites. The calculated Vmax of 4.5 was found in normal subjects.

Reduction of thiamine absorption following parenteral or oral ethanol provides an explanation for the occurrence of thiamine deficiency syndromes in alcoholics despite ingestion of food containing minimum requirements of this vitamin. This finding makes it desirable to have alcoholics ingest thiamine at a time when ethanol is not in body fluids or tissues to insure absorption of this vitamin. Further studies are desirable to pinpoint the mechanism of altered absorption observed following receipt of ethanol. Recent studies suggest direct toxic effects on the gastrointestinal mucosa may be responsible since ethanol in amounts given in our studies will reduce elaboration of gastric and hydrochloric acid and temporarily suppress cell replication (22).

Comment

The unpredictability of thiamine hydrochloride absorption

in the sick and injured has made it desirable to evaluate the therapeutic usefulness of other thiamine moieties such as thiamine propyl disulphide (23), whose absorption is not rate-limited. Thiamine in this form is readily available for tissue use and rapidly corrects symptoms of depletion (25). It thus appears that this or another modification of thiamine which is readily absorbed should replace the hydrochloride moiety for oral therapy in the malnourished alcoholic.

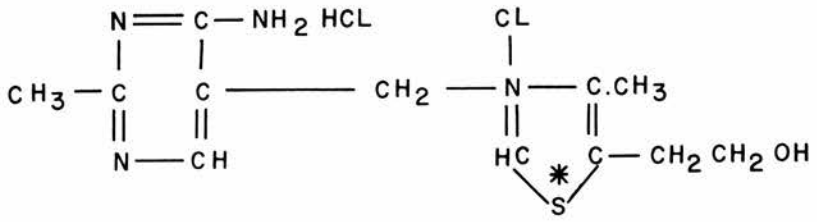
CHAPTER VI

OBSERVATIONS ON THE ABSORPTION AND UTILIZATION OF THIAMINE PROPYL DISULPHIDE

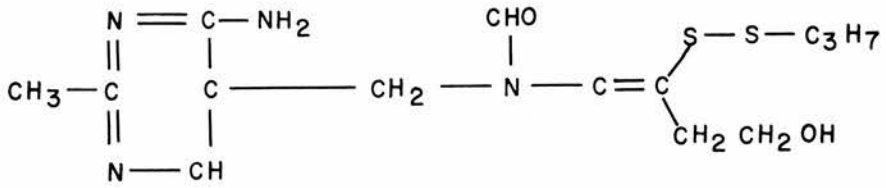
Introduction

Chemical and laboratory evidence of thiamine deficiency often occurs in the alcoholic despite intake of established daily minimal requirements of this vitamin (Thomson, Baker and Leevy, 1968). This could result from failure to absorb thiamine hydrochloride which is usually employed in food or multivitamin supplements. My investigations into the kinetics of 35S-thiamine hydrochloride transport indicate that its absorption is rate-limited, and that either ethanol or dietary deficiency may markedly reduce its intestinal absorption. The reduced absorption in malnourished alcoholics was subsequently confirmed by workers at Johns Hopkins University (Tomasulo, Kater and Iber, 1968) using the test developed earlier in this thesis and published (Thomson, 1966). Japanese workers have found it is possible to achieve higher circulating thiamine levels with thiamine propyl disulphide (Figure 36) than thiamine hydrochloride (Nose and Iwashima, 1965). If ethanol and malnutrition does not interfere with its absorption, it would be desirable to use thiamine propyl disulphide in food or multivitamin supplements to prevent and treat thiamine depletion. The

FIGURE 36. Structural formulae of thiamine hydrochloride (THCl) and thiamine propyl disulphide (TPD).



THIAMINE HYDROCHLORIDE



THIAMINE PROPYL DISULPHIDE

present studies were undertaken to (a) compare the route and kinetics of absorption of thiamine propyl disulphide and thiamine hydrochloride in alcoholic subjects with and without malnutrition; and (b) determine the effectiveness of oral thiamine propyl disulphide in correcting clinical and laboratory evidence of thiamine depletion.

Methods and subjects studied

Studies were conducted in 10 healthy volunteers, 20 alcoholics without stigmata of liver disease or nutritional deficiency, and 35 alcoholics with hepatomegaly with or without neurological abnormalities. All subjects were male with an age range between 31 and 45 years. Laboratory studies included a hematocrit, white blood cell count, blood sugar, blood urea, urinalysis, and serology for syphilis. The alcoholic subjects had an evaluation of hepatic status including a liver biopsy, studies of indocyanine green clearance (Leevy et al., 1967) serum protein electrophoresis, and serum bilirubin. Blood was obtained to measure circulating levels of vitamins A (Carr-Price, 1926), E (Quaife et al., 1949), and C (Schwartz and Williams, 1955) by chemical techniques; and assay of thiamine riboflavin, niacin, folic acid, vitamin B, vitamin B_{12}, biotin, and pantothenic acid, using protozoologic methods (Baker and Frank, 1969). Patients with neurologic signs had a spinal tap with measurement of cerebrospinal

fluid, pyruvate, and lactate. (Friedmann and Haugen, 1943; Olson, 1962).

Healthy volunteers and alcoholics without liver disease had no clinical or laboratory abnormalities. Clinical stigmata of nutritional deficiency were present in each of the alcoholics with hepatomegaly. Eighteen had glossitis, 10 peripheral neuropathy, 5 skin hyperkeratosis, and 6 clasic features of Wernicke's encephalopathy including ocular palsy, confabulation, ataxia, and peripheral neuropathy. None had ascites, jaundice, hepatic coma, or bleeding oesophageal varices. Liver biopsies revealed fatty metamorphosis from 2 plus to 4 plus in 30, and moderately advanced Laennec's cirrhosis in 5 of the patients. Abnormalities of serum protein electrophoresis and indocyanine green removal (Leevy et al., 1967) were present in each of the patients with histological abnormalities. A serum folic acid of less than 4.0 mμgm. per ml. was present in 25, a serum B_6 of less than 28 mμgm. per ml. in 15, blood thiamine of less than 20 mμgm. per ml. in 13, and blood nicotinic acid of less than 2.5 μgm. per ml. in 5 patients.

Comparisons were made of blood and urinary levels of thiamine after receipt of 20 mgm. or 50 mgm. or thiamine propyl disulphide or thiamine hydrochloride given per os after an overnight fast. Subjects were given the other form of thiamine one week later. Ten ml. of blood was drawn from the antecubital vein before thiamine

administration and 1/2, 1, 2, 4, and 24 hours afterwards. Urine was collected for 24 hours before and after the test dose. Ochromonas danica was used to measure thiamine activity in blood and urine (Baker and Frank, 1969).

Protozoological measurement of thiamine activity in blood and urine was measured by the specific and highly sensitive method of Baker et al. (1964) using O. danica. Measurement of metabolically active forms of thiamine in blood and urine following oral administration of equivalent doses of thiamine propyl disulphide or thiamine hydrochloride allowed a direct quantitative comparison to be made of the therapeutically available compounds.

Culture media and conditions of growth. Maintenance and basal media for Ochromonas danics are given in Tables 34 and 35. The basal medium is made up in double strength (twice the amount shown in Table 35.) The organism is grown under constant illumination provided by three 40-watt 'warm-white' fluorescent lights placed at approximately 1.0 m. from the culture. Cultures were maintained by transfering one drop weekly to new maintenance medium contained in 10 ml. screw-capped tubes.

Assay procedure. 2.5 ml. of double strength basal medium is distributed in 25 ml. borosilicate micro-Fernbach flasks provided with glass caps. The solution to be assayed is

Table 34. Maintenance Medium for O. Danica*

Constituent	Amount
Trypticase** (mgm.)	200
Yeast autolysate***(mgm.)	200
Sucrose (mgm.)	1,000
"1:20 Liver"**** (mgm.)	10
Glycerol (w/v) (mgm.)	500
Distilled water to (ml.)	100

*pH adjusted to 5.0 (KOH or H_2SO_4).

**Baltimore Biologic Laboratories, Baltimore, Maryland.

***Albimi Laboratories, Brooklyn, New York.

****Nutritional Biochemicals Co., Cleveland, Ohio.

Table 35. Basal Medium for O. Danica in Thiamine Assay*

Constituent	Amount
Nitrilotriacetic acid** (mgm.)	20
KH_2PO_4 (mgm.)	30
$3MgCO_3$-Mg $(OH)_2$-$3H_2O$ ("basic $MgCO_3$") (mgm.)	40
$CaCO_3$ (mgm.)	5
Metals mix*** (mgm.)	1
NH_4Cl (mgm.)	50
$MgSO_4$-$7H_2O$ (mgm.)	100
Biotin (mgm.)	0.001
L-Glutamic acid (mgm.)	300
L-Histidine HCl-H_2O (mgm.)	40
L-Arginine HCl (mgm.)	40
Glucose (mgm.)	1,000
Distilled water**** (ml.)	100

*pH adjusted to 5.0 (KOH) after boiling.

**Eastman Organic Chemicals, Rochester, New York.

***Provides the following: (1) Fe, 0.1 mgm. (as Fe-$(NH_4)_2(SO_4)_2$-$6H_2O$); (2) Zn, 0.05 mgm. (as $ZnSO_4$-$7H_2O$); (3) Mn, 0.025 mgm. (as $MnSO_4$-H_2O); (4) Cu, 0.004 mgm. (as $CuSO_4$-$5H_2O$); (5) Co, 0.005 mgm. (as $CoSO_4$-$7H_2O$); (6) B, 0.005 mgm. (as H_3BO_3); (7) Mo, p.0025 mgm. (as $(NH_4)_6Mo_7O_{24}$-$4H_2O$); and (8) V, 0.0005 mgm. (as Na_3VO_4-$16H_2O$).[13]

****As indicated in text, this medium was prepared in double strength. It can be made in quadruple strength without precipitation.

added, and the volume is brought to 5 ml. with distilled water. The flasks are then autoclaved for thirty minutes at 118° to 121° C. 2 ml. of a five day maintenance culture diluted with 10 ml. of distilled water is used for innoculation; one drop of this solution added to each flask. Incubation is at room temperature for three to five days. Growth is expressed in optical density units measured with a Welch Densichron equipped with a red-sensitive probe.

Standard solution. A concentrated standard solution of thiamine hydrochloride (1.0 mgm. per ml.) is prepared in distilled water. From this solution, dilutions are made with distilled water so that a standard curve is obtained containing 0.1, 0.3, 1, 3, 10, 30, and 100 mμgm. of thiamine per ml. of final medium. A blank of single strength medium is included to estimate carry-over.

Thiamine solutions and basal media are stored at 4° C with a few drops of volatile preservative.

Preparation of blood samples. Each ml. of citrated blood is diluted with 2 ml. of 0.5 mgm. of transaconitic acid in 100 ml. of distilled water with potassium hydroxide added to pH 4.5. This solution is autoclaved for thirty minutes at 118° to 121° C. The debris is centrifuged off; 0.5, 1.0 and 1.5 ml. of supernatant is added to individual flasks containing 2.5 ml. of double strength basal medium.

The volume is then brought to 5 ml. with distilled water. The flasks are then autoclaved for thirty minutes, cooled, inoculated and incubated as described.

Preparation of urine samples. An aliquot of the twenty-four hour urine specimen is diluted 1:5 with buffer and autoclaved for thirty minutes; it is then further diluted 1:10 with distilled water to dilute toxic components. The urine, now diluted 1:50 is assayed as is blood, centrifuged if necessary.

Other circulating vitamin levels. Vitamins were assayed in serum or whole blood using the Carr-Price, 1957, for vitamin A, a modification of the method of Schwarts and Williams, 1955, for vitamin C and vitamin E by a modification of the method of Quaife et al., 1949. The B-complex vitamins were assayed in serum or whole blood by protozoologic techniques using Ochromonas danica for biotin, Tetrahymena pyriformis for riboflavin, nicotinic acid, pantothenic acid and the vitamin B6-complex, Ochromonas malhamensis for vitamin B_{12} and Lactobacillus casei for folic acid (Baker and Sobotka, 1962).

Route of absorption. The route of absorption of the two forms of thiamine was compared in 4 of the patients with

cirrhosis using combined umbilical and hepatic vein catheterization. The blood thiamine was within normal limits at the time of the procedure. Each subject was given 50 mgm. of thiamine hydrochloride or thiamine propyl disulphide through a Rehfuss tube. Hepatic blood flow was evaluated using the Fick principle with indocyanine green (Leevy et al., 1962) as the indicator. Arterial, portal venous, and hepatic venous blood were obtained at 10-minute intervals for thiamine levels.

Absorption in malnourished alcoholics. The influence of hepatic and nutritional abnormalities on intestinal absorption of thiamine hydrochloride and thiamine propyl disulphide was investigated in 10 malnourished alcoholics with fatty liver. Subjects were given 50 mgm. of thiamine hydrochloride or thiamine propyl disulphide, the order being randomized. Subjects were then treated with a nutritious vitamin-supplemented diet for 6 to 8 weeks at which time repeat biopsies were normal and clinical evidence of nutritional deficiency had disappeared. An absorption test using the same form of thiamine as given initially was repeated.

Assessment of therapeutic value of thiamine propyl disulphide. The normal spinal fluid thiamine level was established in 35 normal subjects receiving spinal anaesthesia

for minor operations. The clinical effectiveness of thiamine propyl disulphide was evaluated in 6 patients hospitalized because of Wernicke's encephalopathy. Five healthy subjects served as controls. A comparison was made of the ability of thiamine hydrochloride, thiamine pyrophosphate and thiamine propyl disulphide to correct abducens palsy, confabulation, ataxia, peripheral neuropathy, blood and spinal fluid thiamine defects and elevated serum and spinal fluid pyruvate. The controls and patients with Wernicke's encephalopathy were randomly given 50 mgm. thiamine hydrochloride, thiamine pyrophosphate and thiamine propyl disulphide orally, in a single dose, followed by 50 mgm. of thiamine propyl disulphide, if there was no response to initial therapy.

RESULTS

Healthy volunteers and alcoholics without evidence of disease had a mean blood thiamine of 40.7 $\pm$ 1.9 mμgm. per ml. Patterns of absorption were identical in these groups. Oral administration of 50 mgm. of thiamine propyl disulphide produced a peak blood thiamine of 1000 mμgm. per ml. within 15 to 30 minutes, while 50 mgm. of thiamine hydrochloride caused a maximum blood thiamine of 150 mμgm. per ml. within 1 to 2 hours in the portal vein. A mean of 8.22 $\pm$ 1.16 mgm. of thiamine was excreted over a 24 hour period after receipt of thiamine propyl disulphide as

compared with a mean of 1.36 ± 0.42 mgm. following thiamine hydrochloride (Figure 37). The increase in thiamine first appeared in portal venous blood, secondly, in the hepatic venous blood, and finally, in the femoral arterial blood after either thiamine hydrochloride or thiamine propyl disulphide (Figures 38, 39). Urinary excretion of thiamine during a 24 hour period increased in a linear fashion from 1.86 ± 0.05 mgm. to 7.8 ± 1.3 with administration of 10 to 50 mgm. of thiamine propyl disulphide. Urinary thiamine showed an increase of only 0.22 ± 0.03 mgm. to 1.42 ± 0.25 mgm. after 10 to 50 mgm. of thiamine hydrochloride (Figure 40).

Malnourished alcoholics with fatty liver exhibited an 80 per cent decrease in absorption of thiamine hydrochloride over control values. There was a marked increase in arterial blood thiamine of 200 ± mμgm. per ml. 1 hour after thiamine propyl disulphide, and an increase of only 9.2 ± 3.0 mμgm. per ml. 1 hour after thiamine hydrochloride (Figure 41). Mobilization of liver fat and improvement of nutrition was followed by restoration of normal patterns of intestinal absorption.

Spinal fluid thiamine was 30.5 ± 5.4 mμgm. per ml. in normal subjects. Spinal fluid and blood pyruvate were 0.95 mgm. per cent and 0.76 mgm. per cent, respectively in normal subjects. Administration of 50 mgm. of thiamine

FIGURE 37. Blood thiamine levels and urinary excretion of thiamine following administration of 50 mgm. of thiamine hydrochloride (THCl) or thiamine propyl disulphide (TPD).

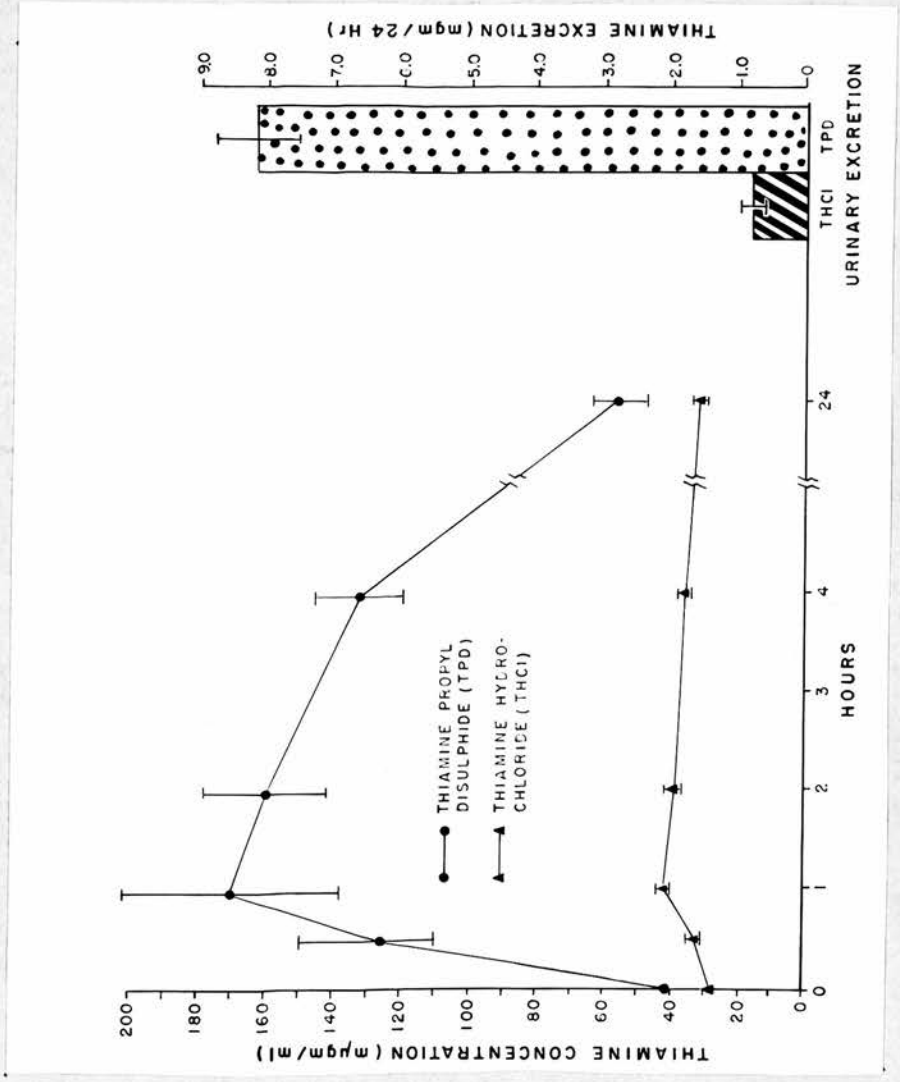

THIAMINE CONCENTRATION (mμgm/ml)
200
180
160
140
120
100
80
60
40
20
0
0
1
2
3
4
24
HOURS
THIAMINE PROPYL DISULPHIDE (TPD)
THIAMINE HYDRO-CHLORIDE (THCl)
URINARY EXCRETION
THIAMINE EXCRETION (mgm/24 Hr)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
THCl
TPD

FIGURE 38. Blood thiamine levels in portal vein, hepatic vein, and femoral artery following 50 mgm. of thiamine propyl disulphide (TPD).

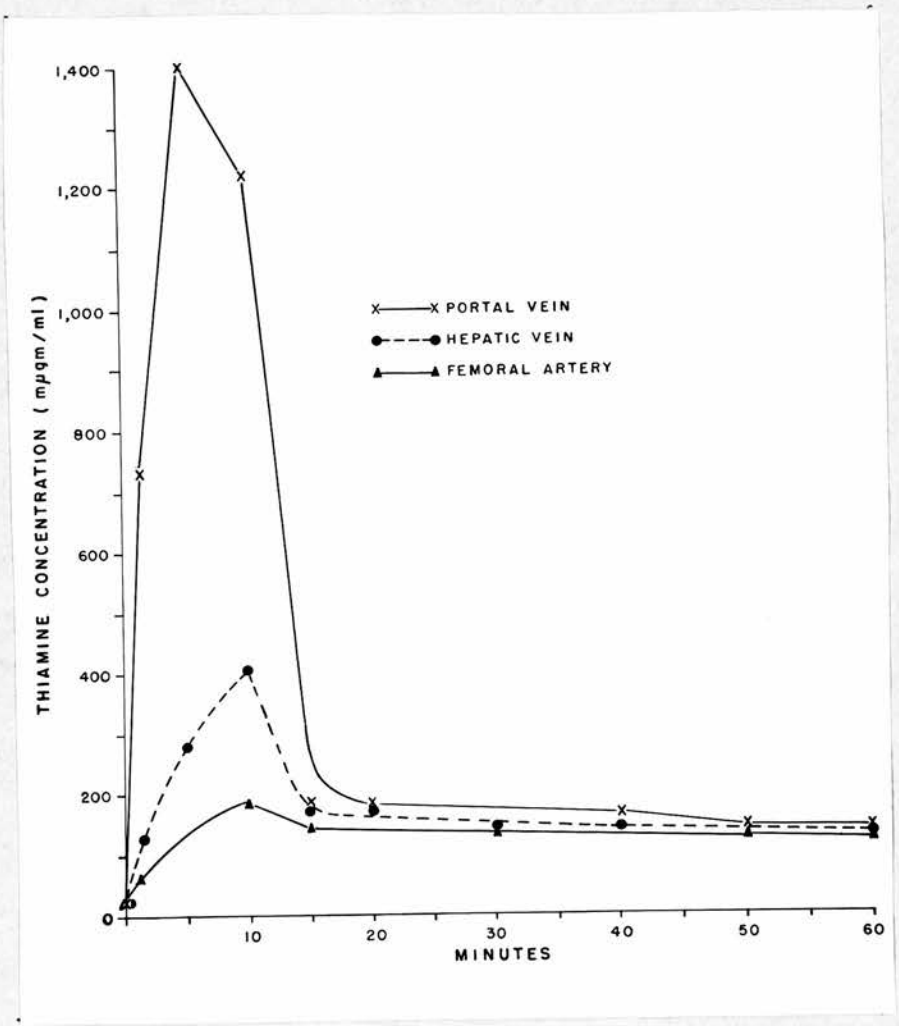

1,400
1,200
1,000
800
600
400
200
0
THIAMINE CONCENTRATION (mμgm/ml)
PORTAL VEIN
HEPATIC VEIN
FEMORAL ARTERY
10
20
30
40
50
60
MINUTES

FIGURE 39. Blood thiamine levels in portal vein, hepatic vein and femoral artery following 50 mgm. of thiamine hydrochloride (THCl) orally.

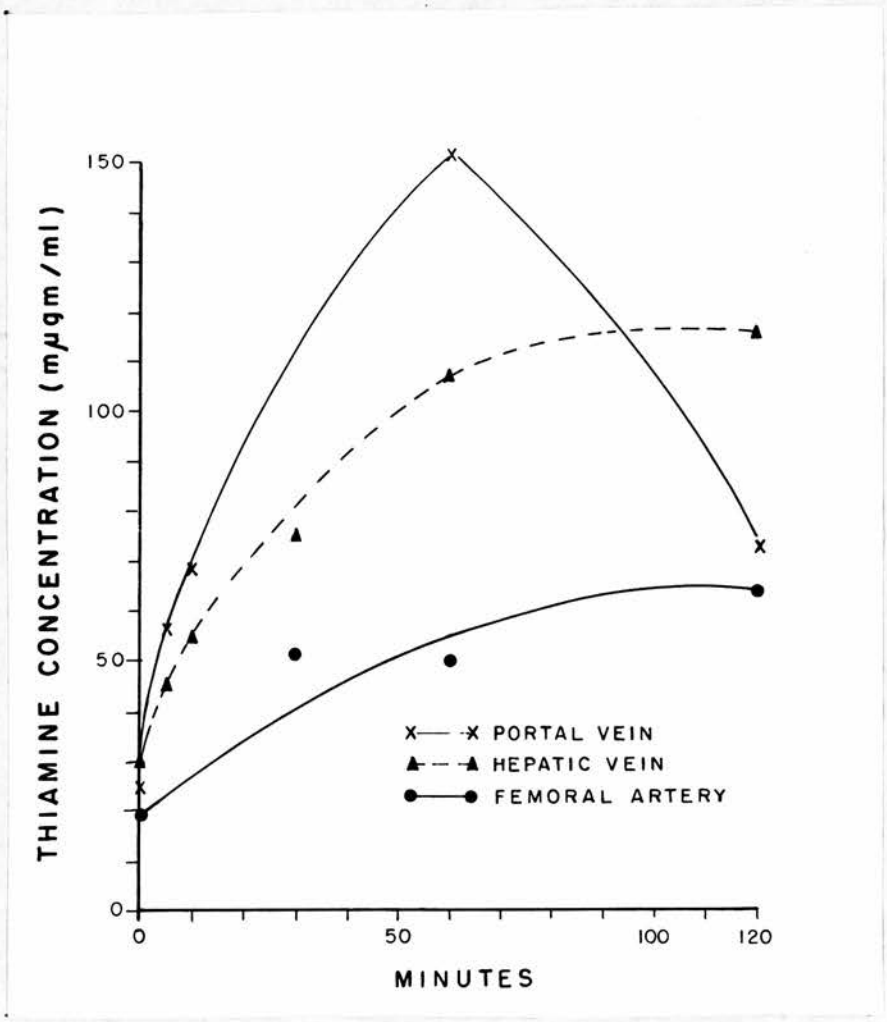

150
100
50
0
THIAMINE CONCENTRATION (mμgm/ml)
0
50
100
120
MINUTES
PORTAL VEIN
HEPATIC VEIN
FEMORAL ARTERY

FIGURE 40. Relationships between the amount of oral administration of thiamine hydrochloride (THCl) or thiamine propyl disulphide (TPD) and the urinary excretion of thiamine.

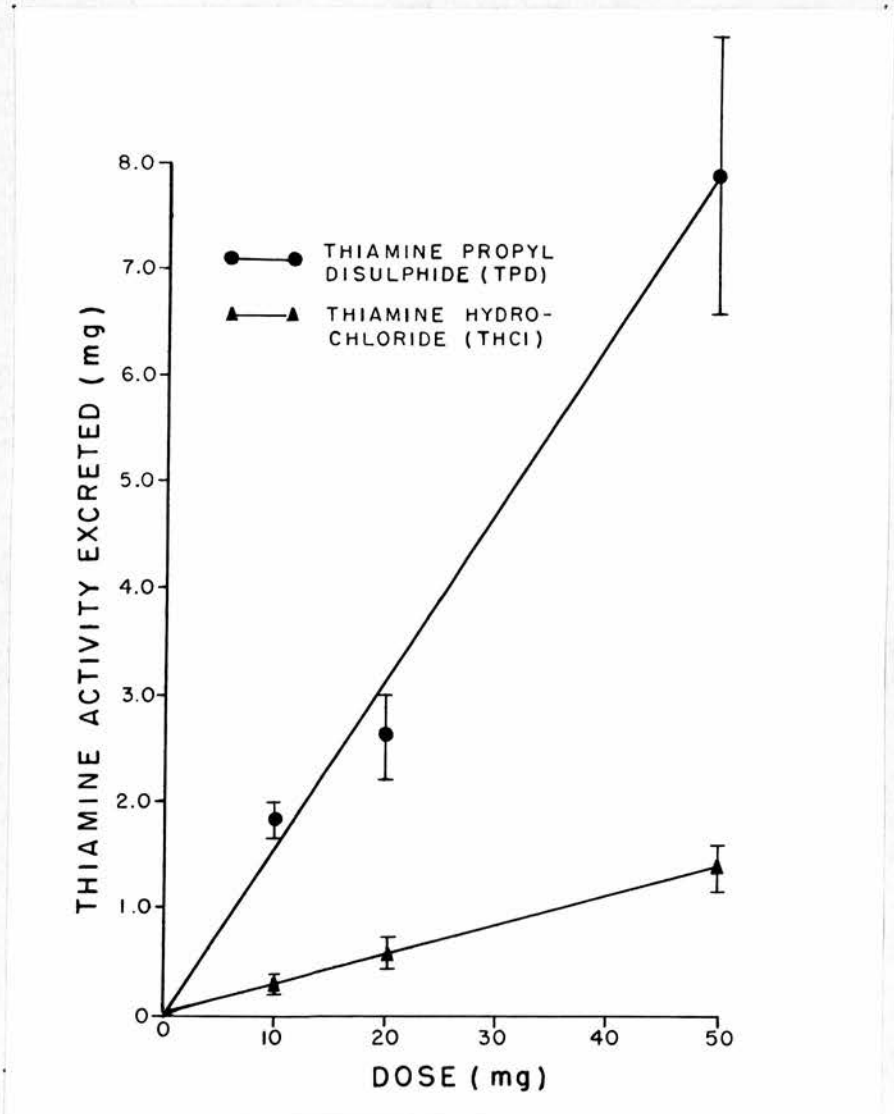
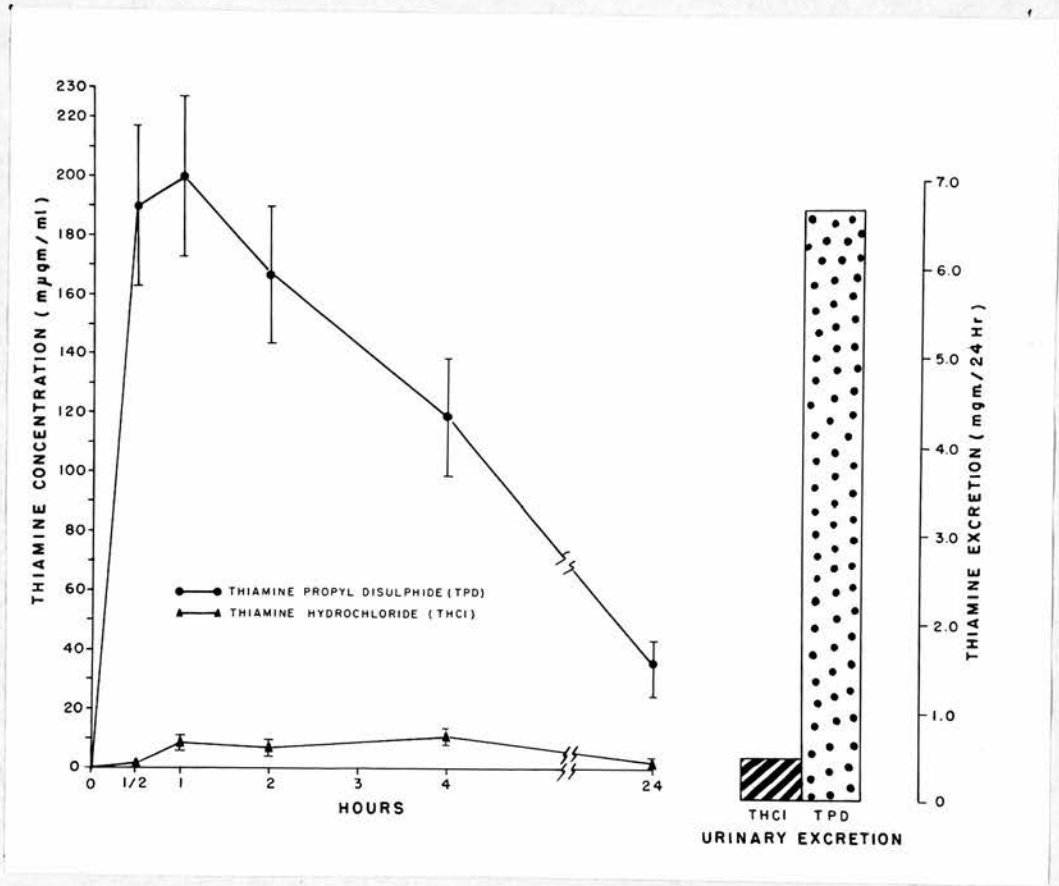

THIAMINE ACTIVITY EXCRETED (mg)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
THIAMINE PROPYL DISULPHIDE (TPD)
THIAMINE HYDRO-CHLORIDE (THCl)
0
10
20
30
40
50
DOSE (mg)

FIGURE 41. Increase in blood thiamine and urinary excretion of thiamine in malnourished alcoholic patients given 50 mgm. of thiamine hydrochloride (THCl) or thiamine propyl disulphide (TPD) orally.


THIAMINE CONCENTRATION (mμgm/ml)
230
220
200
180
160
140
120
100
80
60
40
20
0
0
1/2
1
2
3
4
24
HOURS
THIAMINE PROPYL DISULPHIDE (TPD)
THIAMINE HYDROCHLORIDE (THCl)
THCl
TPD
URINARY EXCRETION
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
THIAMINE EXCRETION (mgm/24 Hr)

hydrochloride produced negligible change in cerebral spinal fluid thiamine in deficient subjects, whereas, a 2 to 30-fold increase was noted when thiamine propyl disulphide was given (Figure 42). Transfer of thiamine into the cerebral spinal fluid was rate-limited, requiring 5 to 6 hours for significant increase. The 6 patients with Wernicke's encephalopathy had a mean blood thiamine of 6.4 $\pm$ 0.98 mμgm. per ml. a mean spinal fluid thiamine of 3.2 $\pm$ 1.2 mμgm. per ml. a mean blood pyruvate of 3.11 $\pm$ 0.86 mgm. per 100 ml., and a mean spinal fluid pyruvate of 2.20 $\pm$ 0.28 mgm. per 100 ml. Oral thiamine hydrochloride and thiamine pyrophosphate did not produce a significant change in blood or spinal fluid thiamine or pyruvate and did not improve ocular palsy, confabulation, ataxia or peripheral neuropathy (Table 36). In contrast, the administration of thiamine propyl disulphide either as the initial agent or the second agent, with failure to respond to other oral thiamine preparations, caused a rapid increase in blood and spinal fluid thiamine, reduction in pyruvate and disappearance of ocular palsy (Figure 43, 44, 45). Following such therapy confabulation and other mental abnormalities disappeared in 3, signs of ataxia and peripheral neuropathy disappeared in 3.

Thiamine depletion represents the second most common vitamin deficiency in chronic alcoholics (Leevy et al., 1969),

FIGURE 42. Cerebrospinal fluid thiamine concentrations in control and deficient subjects before and six hours after 50 mgm. of thiamine hydrochloride (THCl) or thiamine propyl disulphide (TPD).

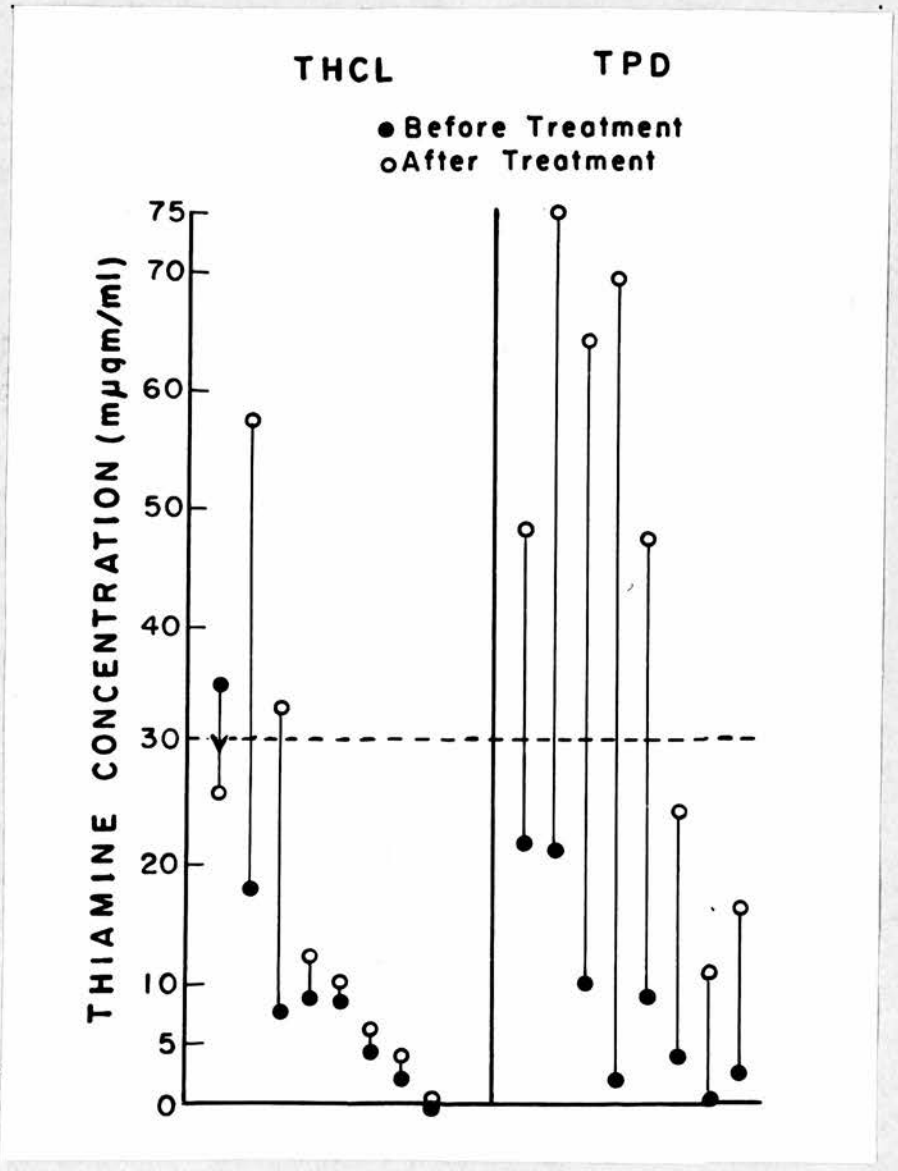

THCL
TPD
● Before Treatment
○ After Treatment
THIAMINE CONCENTRATION (mμgm/ml)
75
70
60
50
40
30
20
10
5
0

TABLE 36

Concentration of thiamine and pyruvate in blood and cerebrospinal fluid (CSF) before and after 50 mgm. of thiamine hydrochloride (THCl) or thiamine propyl disulphide (TPD) orally in six patients with Wernicke encephalopathy.

SUBJECT	ADMISSION			6 HOURS AFTER THIAMINE HYDROCHLORIDE			24–72 HOURS AFTER THIAMINE HYDROCHLORIDE			6 HOURS AFTER THIAMINE PROPYL DISULFIDE		
	Thiamine	Pyruvate	Occular Palsey	Thiamine	Pyruvate	Occular Palsey	Thiamine	Pyruvate	Occular Palsey	Thiamine	Pyruvate	Occular Palsey
BLOOD LEVELS												
SS.	10.0	2.51	Present	12.0	2.44	Present	12.0	2.44	Present	230	2.26	Absent
RH.	8.0	7.64	Present	8.0	2.56	Present	8.0	2.56	Present	177	2.08	Absent
TE.	4.0	1.94	Present	5.0	1.87	Present	5.3	2.42	Present	375	1.63	Absent
CC.	7.8	1.06	Present	← ONLY GIVEN THIAMINE PROPYL DISULFIDE →						99	1.56	Absent
MN.	5.2	3.07	Present	← ONLY GIVEN THIAMINE PROPYL DISULFIDE →						80	1.31	Improved
NW.	3.5	2.42	Present	← ONLY GIVEN THIAMINE PROPYL DISULFIDE →						80	2.00	Absent
CSF LEVELS												
SS.	9.0	1.80	Present	10.0	1.78	Present	10.0	1.78	Present	64	1.20	Absent
RH.	0.0	2.72	Present	0.0	2.08	Present	2.0	2.96	Present	69	1.88	Absent
TE.	3.7	2.02	Present	4.0	1.88	Present	4.3	1.59	Present	52	1.39	Absent
CC.	4.0	1.42	Present	← ONLY GIVEN THIAMINE PROPYL DISULFIDE →						27	1.09	Absent
MN.	0.0	3.46	Present	← ONLY GIVEN THIAMINE PROPYL DISULFIDE →						10	1.72	Improved
NW.	2.6	1.74	Present	← ONLY GIVEN THIAMINE PROPYL DISULFIDE →						16	1.40	Absent

FIGURE 43. Blood thiamine and pyruvate levels in a patient with Wernicke's encephalopathy given 50 mgm. of thiamine hydrochloride (THCl) orally initially and 50 mgm. of thiamine propyl disulphide orally after 24 hours.

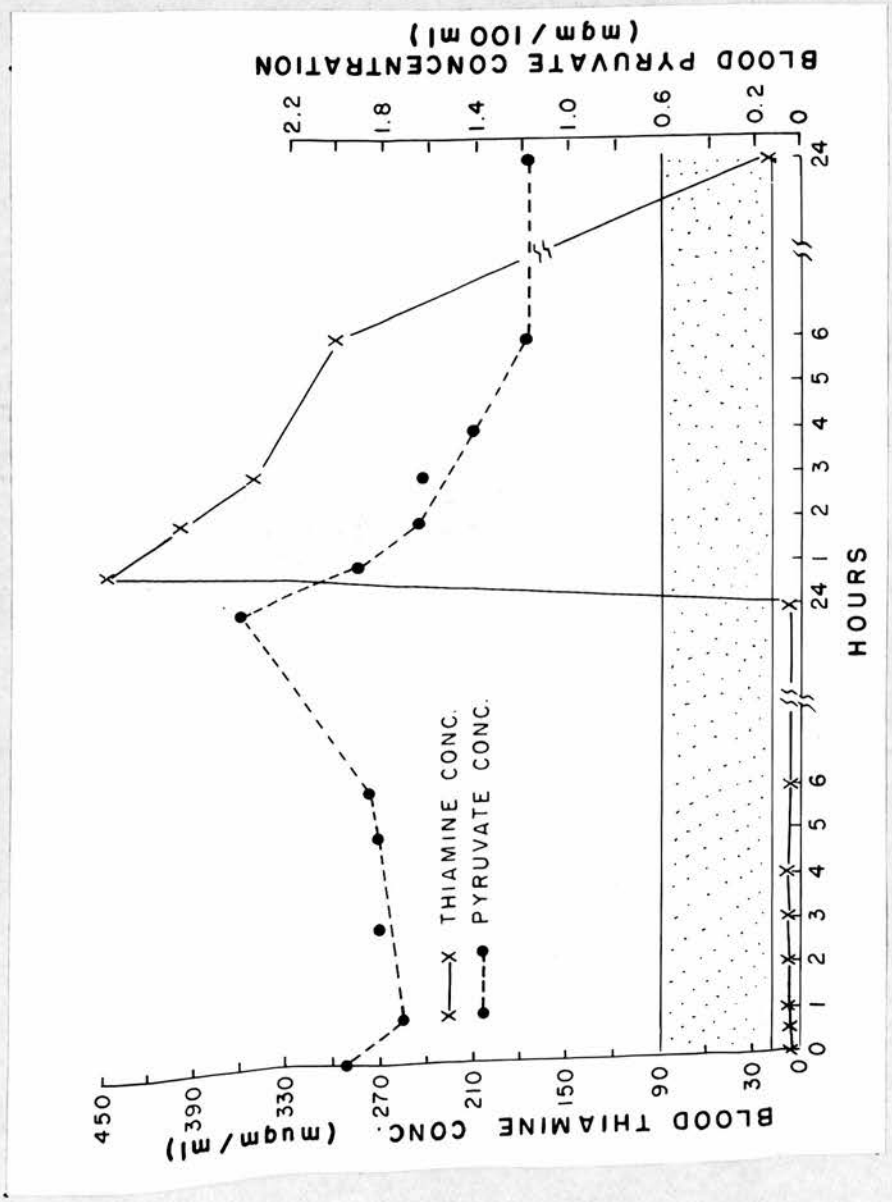

BLOOD PYRUVATE CONCENTRATION
(mgm / 100 ml)
2.2
1.8
1.4
1.0
0.6
0.2
0
24
BLOOD THIAMINE CONC. (mμgm / ml)
450
390
330
270
210
150
90
30
0
X—X THIAMINE CONC.
●---● PYRUVATE CONC.
0 1 2 3 4 5 6
24 1 2 3 4 5 6
HOURS

FIGURE 44. Cerebrospinal fluid thiamine, lactate and pyruvate levels in a patient with Wernicke's encephalopathy given 50 mgm. of thiamine pyrophosphate (TPP) orally initially and 50 mgm. of thiamine propyl disulphide (TPD) orally after 46 hours.

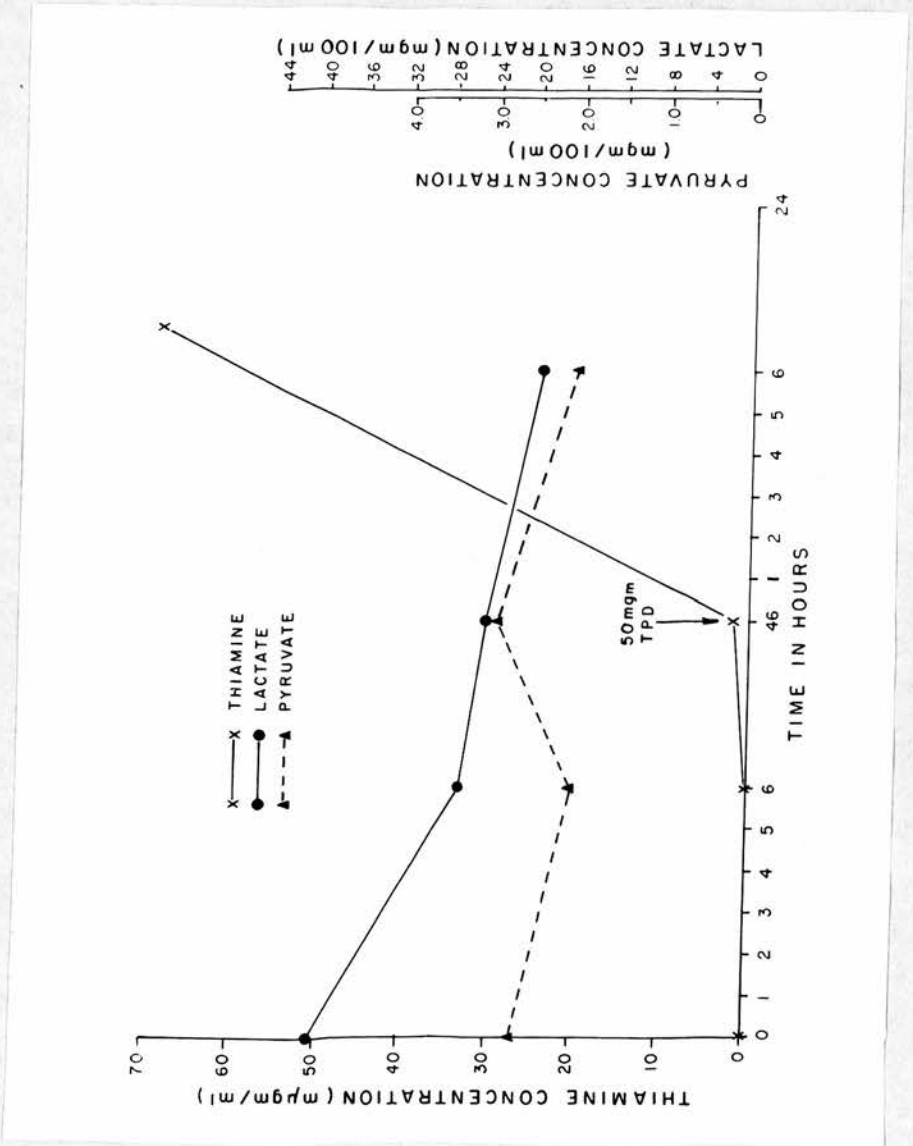

THIAMINE CONCENTRATION (mμgm/ml)
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6
4 6
1 2 3 4 5 6 24
TIME IN HOURS
50mgm TPD
X THIAMINE
LACTATE
PYRUVATE
PYRUVATE CONCENTRATION (mgm/100ml)
4.0
3.0
2.0
1.0
0
LACTATE CONCENTRATION (mgm/100ml)
44 40 36 32 28 24 20 16 12 8 4 0

FIGURE 45. Blood thiamine and pyruvate levels in a patient with Wernicke's encephalopathy given 50 mgm. of thiamine propyl disulphide (TPD) orally on admission.

THIAMINE CONCENTRATION (mμgm/ml)
240
220
200
180
160
140
120
100
80
60
40
20
0
PYRUVATE CONCENTRATION (mgm/100ml)
1.6
1.2
0.8
0.4
0
0
1
2
3
4
5
6
24
TIME IN HOURS
THIAMINE
PYRUVATE

randomly selected hospitalized patients (Leevy, et al., 1965) and pregnant women (Thomson, et al., 1969). Only folic acid which has not been widely used as a supplement because of law or precedent is more commonly deficient. In some instances, deficiency syndromes result from inability to convert thiamine into its metabolically active form because of liver disease (Fennelly et al., 1967) or nucleic acid abnormalities (Leevy, et al., 1969). Most patients with thiamine depletion have not ingested adequate thiamine or fail to absorb sufficient amounts of the vitamin to care for increased needs imposed by tissue injury. Availability of a protozoologic method which provides a reliable measure of the active form of thiamine in biologic fluids and tissues makes it possible to study the effectiveness of the various congeners of this vitamin in clinical practice.

DISCUSSION

Matsokawa and Kawasaki introduced three alli-thiamines: thiamine allyl disulphide, thiamine methyl disulphide and thiamine propyl disulphide, each of which produces significantly higher blood and urine levels than thiamine hydrochloride or thiamine nitrate (Matsokawa and Kawasaki, 1952). Thiamine propyl disulphide has been the most satisfactory of the alli-thiamines (Shimazono and Katsura, 1965). It was found that oral thiamine propyl

disulphide is absorbed more readily with a lower faecal loss than occurs with thiamine hydrochloride. Moreover, following its oral administration, a significantly higher level was found in the blood and liver with ready entry into other body fluids and tissues (Takenouchi and Aso, 1964).

35S-thiamine propyl disulphide experiments indicate that following its intravenous administration thiamine propyl disulphide splits into thiamine sulphide within blood cells, the latter escapes into the plasma and is bound to albumin (Fajiwara, et al., 1964). In vitro studies suggest the same mechanism occurs in intestinal transport and that thiamine propyl disulphide is converted into thiamine and then cocarboxylase (Hioco, et al., 1959). Thiamine propyl disulphide has the disadvantage of being less water-soluble than thiamine hydrochloride (Matsukawa, et al., 1962).

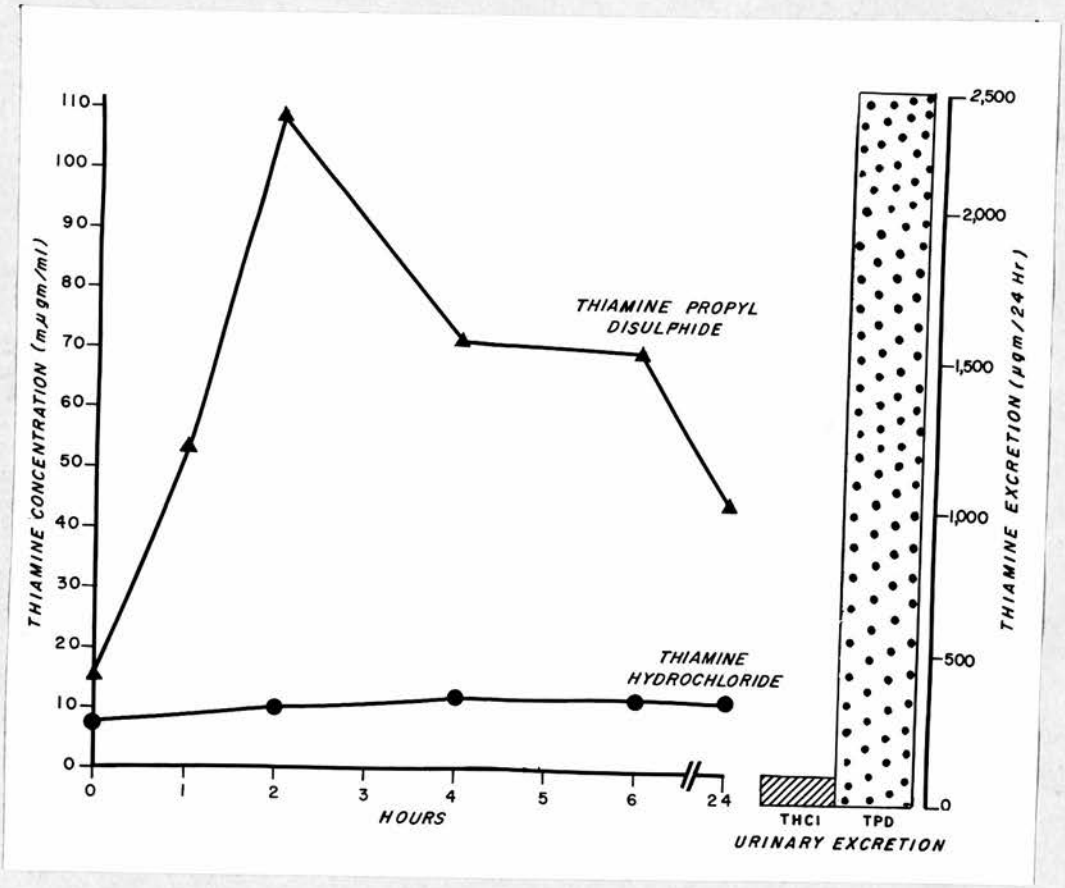
The present study demonstrates that orally administered thiamine propyl disulphide produces a significantly higher blood and urinary level of thiamine than thiamine hydrochloride in malnourished alcoholics. Previous studies using 35S-thiamine hydrochloride indicate that its intestinal absorption may be described by the Michaelis-Menten kinetics (Michaelis and Menton, 1913). Blood levels and urinary excretion of thiamine, after receipt of thiamine

propyl disulphide in the present study increased in a linear fashion with larger oral doses without limitation to absorption over the dose range tested.

The absorption of both forms of thiamine were markedly diminished in malnourished alcoholic patients with fatty liver; however, absorption of thiamine propyl disulphide far exceeded that of thiamine hydrochloride as judged by blood and urinary thiamine levels. Low levels of thiamine often persisted under these circumstances in patients receiving thiamine hydrochloride, however, thiamine propyl disulphide absorption was consistently adequate to correct the thiamine deficit. This was best demonstrated in subjects with Wernicke's encephalopathy in whom thiamine hydrochloride or thiamine pyrophosphate failed to increase either blood or spinal fluid thiamine while equimolar quantities of thiamine propyl disulphide rapidly corrected deficits. Repeated doses of thiamine hydrochloride could prevent a deficiency state or eventually correct body deficits, however, this varies considerably and cannot be relied upon in the alcoholic (Figure 46).

Thiamine propyl disulphide has been used widely in Japan; its administration over periods of months to years has not been associated with any untoward effects. No ill effects were encountered when we gave this drug to normal volunteers or malnourished alcoholics with and without

FIGURE 46. Blood and urinary thiamine concentration in a deficient patient given 20 mgm. of thiamine hydrochloride (THCl) orally initially and 20 mgm. of thiamine propyl disulphide (TPD) orally after 24 hours.


THIAMINE CONCENTRATION (mμgm/ml)
110
100
90
80
70
60
50
40
30
20
10
0
THIAMINE PROPYL DISULPHIDE
THIAMINE HYDROCHLORIDE
0
1
2
3
4
5
6
24
HOURS
THIAMINE EXCRETION (μgm/24 Hr)
2,500
2,000
1,500
1,000
500
0
THCl
TPD
URINARY EXCRETION

symptomatic thiamine deficiency for periods up to 3 months. Oral thiamine propyl disulphide rapidly corrected biochemical and clinical abnormalities due to thiamine depletion. It thus appears that this form of thiamine is valuable for prevention and treatment of thiamine deficiency states.

Pauling, 1968, has postulated that cerebral avitaminosis without overall body depletion may cause mental disfunction. No evidence was found to support this hypothesis for thiamine and reduced amounts of this vitamin in the CSF. appears to be a reflection of body depletion of thiamine. Neurologic disease attendant to a deficiency of thiamine is a common complication of alcoholism which could be prevented in part by use of readily absorbable forms of this vitamin. There are several alli-thiamine homologues and S-acyl-thiamine derivatives which are more readily absorbed from the intestine and penetrate red cells and tissues with greater facility than thiamine hydrochloride. Wide use of a more absorbable form of thiamine in pretzels and other carbohydrates on which alcoholics and many impoverished people subsist could reduce greatly the incidence and sequelae of thiamine depletion. Consideration should, therefore, be given to substitution of thiamine propyl disulphide or a similar preparation for thiamine hydrochloride as a food supplement and in multivitamin capsules.

CHAPTER VII

GENERAL DISCUSSION

Evolution has made animals dependent upon environmental supplies of vitamins. Natural changes in the surroundings or modifications imposed by man may limit their supply. Disease processes or orally administered agents may interfere with specialised mechanisms developed for their absorption or prevent adequate utilization imposing serious physical and mental limitations upon the individual. Investigations were undertaken to examine the critical role of intestinal absorption in determining the thiamine status in man. It was thought that the behaviour of thiamine may be representative of other rate limited nutrients.

The measurement of absorption in man must often be indirect and it is difficult to separate the processes of utilization and storage from absorption. Additional problems in assessing thiamine absorption have been due to an inability to measure thiamine and its metabolites accurately. Investigations using 35S-thiamine hydrochloride indicate that radioactive thiamine can be used to study the absorption of this vitamin in man. It is evident that sufficient thiamine can be absorbed to meet

daily requirements in the presence of adequate intake of other nutrients and normal liver function. The absorption of thiamine hydrochloride is limited. The existence of a special mechanism for absorption of a substance so highly diffusible suggests that it has developed to exclude excessive amounts of thiamine rather than to ensure adequate absorption. Failure of this mechanism may occur because of destruction of these sites as in primary malabsorptive disease (ideopathic steatorrhoa), may result from ethanol ingestion or malnutrition and liver disease. Absorption is corrected when the pathological process returns to normal or following the cessation of ethanol. However, during the period when these patients have the greatest need to replace their depleted stores and repair damaged tissues, they are unable to absorb thiamine hydrochloride adequately. The use of thiamine propyl disulphide (TPD), which is absorbed by simple diffusion, allows us to overcome the block imposed by the intestine and provides an adequate supply of the metabolically active form of this vitamin.

These investigations have been limited to the absorption of only one of the vitamins. Information on the influence of disease on the absorption of other vitamins in man or of the inter-relationship of one vitamin upon another, is extremely limited. The incidence of vitamin

deficiency is only now being recognized and its full effect on the development and efficiency of intellectual and physical abilities is not known. If man is to succeed in remodelling his environment, future research must investigate and solve these problems.

SUMMARY OF THESIS

A method has been developed to measure vitamin B_1 absorption in man by measuring urinary and serum radioactivity following an oral dose of radioactive 35S-thiamine hydrochloride given along with a parenteral injection of non-radioactive thiamine. Analysis of small intestinal juice and characterization of radioactive material in the urine indicated that little breakdown of thiamine occurred in the intestine or during its passage through the body under the conditions of the test. The amount absorbed, urinary and serum patterns of radioactivity were established in control subjects. Studies using combined umbilical and hepatic vein catheterization indicated that thiamine, which was absorbed high in the gastro-intestinal tract, initially appeared in the portal blood and subsequently in the hepatic venous and arterial blood. Mathematical formulations used to describe enzyme-substrate interactions (Michaelis-Menton) were found applicable to thiamine hydrochloride absorption. Based on 72 hour urinary excretion, normal subjects had a mean Vmax of 4.2 mgm. in comparison with a Vmax of 1.1 mgm. in a patient with intestinal resection. Old age was not associated with an inevitable reduction in absorption and deficient circulating levels of thiamine could be avoided if the diet provided the recommendable allowance.

Patients with primary malabsorptive disease (ideopathic steatorrhoea) exhibited a significant reduction in intestinal absorption of 35S-thiamine which returned to normal following treatment with a gluten free diet. Investigations in malnourished alcoholics demonstrated a marked decrease in thiamine hydrochloride absorption in the absence of steatorrhoea, radiologic abnormalities or significant histological changes in the jejunum, attributed to a decrease in the number of receptor sites available. The block of absorption demonstrated to be at the intestinal level only returned to normal following receipt of a high protein-vitamin supplemented diet for 6 to 8 weeks. Ethanol (1.5 gm/kgm.) given either parenterally or orally, caused a 50% reduction in thiamine absorption in 4 or 12 healthy subjects providing an explanation for the occurrence of thiamine deficiency syndromes despite ingestion of food containing minimum requirements of this vitamin.

The absorption of thiamine propyl disulphide (TPD) was investigated using a protozoologic technique (Ochromonas danica). The route of absorption was shown also to be via the portal vein but the absorption was not found to be rate limited. This permitted the intestinal block imposed by malnutrition to be effectively bypassed. Cerebrospinal (CSF) and blood thiamine levels were measured in normal subjects and in patients with symptomatic thiamine depletion. Results indicated that reduced amounts of thiamine in CSF

was a reflection of body depletion of the vitamin. When congeners of thiamine with different membrane transport capacity (-hydrochloride (THCl), -propyldisulphide (TPD), and -pyrophosphate (TPP)) were used for repletion studies inadequate intestinal absorption of THCl and TPP failed to correct thiamine deficiency or reverse the neurological signs of Werneke's encephalopathy. Thiamine propyl disulphide (TPD) consistently produced high blood and CSF levels after oral administration. It was shown to be metabolically effective by relieving the symptoms of encepholopathy and exhibited no untoward effects. Because of the unpredictability of thiamine hydrochloride (THCl) absorption in the sick and injured it was recommended that thiamine propyl disulphide (TPD) or another modification of thiamine which is readily absorbed should replace the hydrochloride moiety for food supplementation and oral therapy in the malnourished alcoholic.

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COMMUNICATIONS

In connection with the experimental work reported in this thesis it was considered desirable to make the following communications to learned societies and journals.

COMMUNICATIONS TO LEARNED SOCIETIES

(1) 'Studies on the absorption of radioactive sulphur-labelled thiamine hydrochloride in man'. A. D. Thomson. Medical Research Society - London, January, 1966.

(2) 'Factors influencing the excretion of oral test doses of thiamine in human subjects'. A. D. Thomson. Edinburgh Pathology Society - Edinburgh, February, 1966.

(3) 'Absorption of thiamine in old age'. A. D. Thomson. British Geriatric Society - Edinburgh, April, 1966.

(4) 'Thiamine absorption in alcoholism'. A. D. Thomson, H. Baker and C. M. Leevy. American Society Clinical Nutrition - Atlantic City, May, 1968.

(5) 'Role of malabsorption in thiamine deficiency'. A. D. Thomson, H. Baker and C. M. Leevy. 2nd. Western Hemisphere Nutrition Congress, San Juan, Puerto Rico, August, 1968.

PUBLICATIONS

(1) 'The absorption of sulphur-labelled thiamine hydrochloride in control subjects and in patients with intestinal malabsorption'. A. D. Thomson. Clin. Sci. 1966, 31, 167.

(2) 'Thiamine absorption in old age'. A. D. Thomson. Geront. Clinica, 1966, 8, 354.

(3) 'Thiamine absorption in alcoholism'. A. D. Thomson, H. Baker, and C. M. Leevy. Am. J. Clin. Nutr., 1968, 21, 537.

(4) 'Pattern of 35S-thiamine hydrochloride absorption in the malnourished alcoholic' - in preparation.

(5) 'Observations on the absorption and utilization of thiamine propyl disulphide' - in preparation.

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THE ABSORPTION OF RADIOACTIVE SULPHUR-LABELLED THIAMINE HYDROCHLORIDE IN CONTROL SUBJECTS AND IN PATIENTS WITH INTESTINAL MALABSORPTION

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SUMMARY

1. A method is described for investigating vitamin B_1 absorption in man by measuring the urinary radioactivity during the 24 hr following an oral dose of radioactive [^{35}S]thiamine which is given along with a parenteral injection of non-radioactive thiamine hydrochloride.

2. Analysis of small intestinal juice and characterization of radioactive material in the urine indicate that the urine contains radioactive thiamine which was unchanged prior to absorption.

3. In control subjects, the major period of thiamine absorption occurred within the first 2 hr and practically all of the oral dose absorbed was excreted during the first 24 hr.

4. Eight patients with untreated primary malabsorptive disease (idiopathic steatorrhoea) showed a mean urinary excretion of radioactive thiamine significantly less than in the control group. The rate of excretion was markedly reduced but the period of maximal excretion occurred at approximately the same time as in control subjects. Thirteen patients with treated primary malabsorptive disease showed no significant difference from the control group.

5. Ten patients who had undergone gastric surgery and two with resection of the terminal ileum excreted amounts which did not differ from the control group. Normal results were also found in four patients with pernicious anaemia.

Disease of the small intestine may cause impaired absorption of certain water-soluble vitamins. Patients with primary malabsorptive disease (idiopathic steatorrhoea), for example, frequently fail to absorb folic acid normally (Girdwood, 1953; Chanarin, Anderson & Mollin, 1958;

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Cox *et al.*, 1958; Girdwood & Delamore, 1961). Although clinical evidence of deficiency of other water-soluble vitamins is rarely present, investigations using the tryptophan loading test have suggested subclinical deficiency of pyridoxine in patients with tropical sprue and primary malabsorptive disease (Kowlessar *et al.*, 1961; Sigler *et al.*, 1962). Baker & Sobotka (1962) have shown that patients with primary malabsorptive disease may have subnormal levels of serum pyridoxine and Brain & Booth (1964), using tritium-labelled pyridoxine, have demonstrated impaired absorption of pyridoxine in some of these patients.

Girdwood (1956), using microbiological methods, studied the absorption of water-soluble vitamins in patients with primary malabsorptive disease, but found no evidence of malabsorption of thiamine, riboflavin or pyridoxine in ten patients with impaired absorption of folic acid. Except for this investigation, little has been published on the absorption of thiamine in patients with intestinal malabsorption.

The availability of ^{35}S-labelled thiamine has allowed some of the limitations of the microbiological methods to be overcome. This paper presents a method for the measurement of thiamine absorption using radioactive thiamine and compares the results found in control subjects with those obtained in various groups of patients with gastro-intestinal disorders.

MATERIALS AND METHODS

Materials used

All of the thiamine preparations were supplied as the hydrochloride. ^{35}S-labelled thiamine was obtained from the Radiochemical Centre, Amersham. The radioactive material used orally had a specific activity of 177 mCi/g and was found to be radiochemically pure when tested in the three chromatographic systems described below. The solid material was dissolved in distilled water, 100 μCi (ten doses) dispensed into each ampoule, freeze dried and stored at $-20°$. It was reconstituted by adding 10 ml distilled water. In a few experiments, radioactive thiamine was used intravenously. This material was supplied in aqueous solution, pH 5; it had a specific activity of 12·0 μCi/ml and analysis showed that it was more than 90% radiochemically pure. The non-radiocative thiamine was supplied in ampoules containing 100 mg in 1 ml by Roche Products Ltd, England.

Method for the study of absorption of ^{35}S-labelled thiamine

The radioactive material was diluted with non-radioactive thiamine so that each test dose contained 1·0 mg and 10 μCi of radioactivity dissolved in 20 ml of water. A quantity of the solution from which the test dose was prepared was used as a standard. The test dose was given orally to subjects after an overnight fast, and in some experiments a parenteral injection of non-radioactive thiamine was given immediately before the oral dose. The standard test referred to later includes both a 1·0 mg oral dose of radioactive thiamine and a parenteral injection of 200 mg non-radioactive thiamine given at the same time as the oral dose. Absorption was assessed by measuring the urinary radioactivity during the following 24 hr. The urine collections were removed after the intervals 0–5, 5–12 and 12–24 hr, the volume measured and a sample stored at $-20°$ until the radioactivity was measured. The urine samples were counted in an Isotope Developments Ltd, Reading, Berkshire, liquid scintillation counter type 6012, using 0·8 g/100 ml of 2-5-diphenyloxazole and 0·005 g/100 ml of 1,4-bis(2[5-phenyloxazolyl]) benzene dissolved in toluene as the liquid scintillator. The samples were prepared by adding

1·0 ml urine to 3 ml hyamine chloride, 1·5 M solution in methanol, and adding 10 ml liquid scintillator. To each sample, 10 μCi of the test dose was added as an internal standard.

Identity of the radioactivity present in intestinal contents and urine after oral administration of ^{35}S-labelled thiamine. Urine was collected in a brown bottle surrounded by a freezing mixture of ice and solid carbon dioxide (Drikold, I.C.I. Ltd), during the periods 0–1½, 1½–3 and 3–12 hr. Following voiding, the urine was either analysed immediately or stored at −20°. A modification of the phenol extraction procedure of Iacono & Johnson (1957) was used to extract the radio-metabolites from the urine. Over 90% of the radioactivity was removed by this method.

Descending chromatography was performed on Whatman No. 1 paper using n-propanol–water–M-acetate buffer, pH 5 (70 : 20 : 10), the upper phase of n-butanol–acetic acid–water (40 : 10 : 50), and the upper phase of s-butanol–water as solvents. The papers were scanned in a BTL Radioactive Chromatogram Scanner and radioautographs were prepared with Gavert no-screen X-ray film; the film was exposed to the chromatogram for up to 3 months, depending on the amount of radioactivity.

Identification of thiamine compounds on paper chromatograms

A. *Thiochrome test.* After drying the chromatograms at room temperature thiamine and its esters present on the paper were converted to the corresponding thiochromes by the method of Siliprandi & Siliprandi (1954); this test will detect 1·0 μg thiamine.

B. *Use of micro-organisms.* Some chromatograms were not sprayed but were scanned and the radioactivity eluted with 3 ml distilled water. The ability of the eluate to support growth of *O. danica*, which requires intact thiamine (Heinrich, 1955; Baker *et al.*, 1964), was compared with a control consisting of an equivalent area cut from the same chromatogram. The method of Baker *et al.* (1964) was used for thiamine assay. Following incubation, the material was centrifuged at 3000 rev/min for 30 min and subsequently washed by centrifuging after re-suspension in two aliquots of 20 ml sterile distilled water. The radioactivity could not be washed from the organisms. The organisms were finally suspended in gel-scintillator and the radioactivity present counted by well-scintillation counting.

C. *Separation of radioactive compounds.* To confirm that complete separation had been achieved, two chromatograms were run in each of the three solvent systems and the radioactivity corresponding to thiamine eluted as above. Each of the eluates was then re-run in a different chromatographic solvent system and produced a single spot at the same R_F as thiamine. The radioautographs also showed a single discrete spot.

Demonstration of thiamine and its metabolites in the urine. Four patients were given 300 mg of non-radioactive thiamine hydrochloride intravenously 48 hr before the experiment. On the day of the experiment they received the standard test of 1·0 mg oral radioactive thiamine hydrochloride and 200 mg non-radioactive thiamine intravenously. In three subjects, over 90% of the radioactivity excreted during the 0–12 hr period had the same R_F as thiamine in all three solvent systems and gave a positive thiochrome test. The eluted radioactive substances supported the growth of *O. danica* and the radioactivity could not be washed from the cells. In the fourth subject, during the period 1½–3 hr after the dose 90% of the radioactivity was due to thiamine but this fell to 60% in the 3–12 hr period. No attempt was made to identify the other radioactive compounds.

Extraction of radio-metabolites from small intestinal juice. Two normal subjects were intubated using a 1·5 mm bore polyvinyl tube weighted at one end. The duodenal–jejunal junction

is approximately 90 cm and the ileo-caecal valve 350 cm from the nose in adults (Blankenhorn, Hirsch & Ahrens, 1955). Sampling in one subject was from points 105 and 195 cm from the nose on two successive days, i.e. from the jejunum and from the proximal ileum; in the other, sampling was only from the proximal ileum. The fasting subjects were fed a test meal consisting of 'Humanized Trufood' (Trufood Ltd, The Creamaries, Wrenbury, Cheshire) and 5 μCi of [^{35}S]thiamine with sufficient non-radioactive thiamine to give a final thiamine content of 1·0 mg. The small intestinal juice was obtained by continuous sampling into a flask surrounded by a freezing mixture. The volume of intestinal juice decreased considerably after the third hour. Consequently, the observations relate to the 0–3 hr period. The pH of the intestinal juice was measured with a glass electrode and never rose above pH 6·1, i.e. it was always within the range at which thiamine is stable. At the end of the experiment, gastrografin was introduced into the tube to check the position of the sampling point.

Twenty ml of small intestinal juice were dialysed against three 100 ml portions of distilled water left for 24 hr at 4°. The dialysates were freeze-dried and then dissolved in 30 ml absolute ethanol. The solution was concentrated *in vacuo* at 35° to about 4 ml and a sample chromatographed. It was found that over 90% of the radioactivity was identical, chromatographically, with thiamine.

Subjects studied

Controls were sixty-five convalescent, ambulant hospital patients free from gastro-intestinal, endocrine, collagen and malignant diseases. They had normal hepatic and renal function and were not receiving drug therapy. They showed no signs of nutritional deficiency.

Patients with primary malabsorptive disease included ten cases who had not been treated with a gluten-free diet. The diagnosis was established by jejunal biopsy and the results of the following absorption tests—folic acid absorption (Girdwood, 1953; Girdwood & Delamore, 1961), vitamin B_{12} absorption (Schilling, 1953), xylose absorption (Fourman, 1948) and faecal fat excretion (Kamer *et al.*, 1949). A sternal marrow was performed and serum folate, formiminoglutamic acid (FIGLU), and vitamin B_{12} levels were measured. All patients had a barium meal and follow-through examinations. There were also thirteen patients who had been treated with a gluten-free diet for periods varying from 3 months to 5 years.

The other patients included ten who had had gastric surgery and three patients with pernicious anaemia who had confirmed acid-fast achlorhydria in response to a maximum histamine test meal and were untreated at the time of investigation. Three patients had Crohn's disease and one who had had a sub-total proximal gastrectomy with an end-to-side oesophago-gastric anastamosis for an anaplastic carcinoma of the stomach 17 years previously, but there was no post-mortem evidence of a recurrence when she died from a myocardial infarction some months after completion of the studies. Two of the patients with Crohn's disease has had a resection of the terminal iluem and a right hemicolectomy.

RESULTS

Investigation of test conditions.

The excretion of radioactivity when ten control subjects were only given 1·0 mg of radioactive thiamine orally was 6·1% (SEM $\pm$ 0·64) but if the oral dose was preceded by giving

300 mg of non-radioactive thiamine intravenously 48 *hr before*, the excretion of radioactive thiamine rose to 15·3% (SEM ± 2·63). This difference is highly significant ($t = 4{\cdot}26$, $P < 0{\cdot}01$).

The standard test. The effect of varying the size of an intravenous dose of non-radioactive thiamine given *at the same time* as the oral dose of radioactive material was studied and the the optimum amount for this intravenous flushing dose was found to be 200 mg. This dose ensured maximal excretion and was thereafter used in the standard test of thiamine absorption (Table 1). Giving thiamine to control subjects 2 days before they received the standard test did not alter the mean urinary excretion of radioactivity (Table 1) ($t = 0{\cdot}814$, $0{\cdot}2 < P < 0{\cdot}5$).

Fig. 1 shows the cumulative excretion of radioactivity after 200 mg flushing dose in a control subject who received 300 mg thiamine intravenously 48 hr before. Excretion was maximal

TABLE 1. Twenty-four hour urinary excretion of radioactive thiamine by normal subjects: The effect of injecting varying amounts of non-radioactive thiamine at the time of administering 1·0 mg radioactive thiamine by mouth on the excretion of radioactive thiamine in the urine

Intravenous dose of non-radioactive thiamine (mg)	Urinary excretion of radioactivity (% oral dose*)	
	Before loading	After loading†
0	6·1 ± 0·64 (10)	15·3 ± 2·63 (6)
25		35·0 ± 2·95 (5)
50		39·5 ± 3·18 (7)
100		51·7 ± 5·04 (9)
200	48·2 ± 4·54 (10)	54·1 ± 5·66 (10)
300		50·2 ± 3·66 (8)

* The results are expressed as the mean ± SEM (*n*). Urine was collected for 24 hr.

† Subjects given 300 mg thiamine i.v. 48 hr before test, and a further dose of thiamine at the time of the test.

between 1–2 hr during which time 41% of the dose was excreted. By 12 hr, excretion was almost complete, 3% being added in 12–24 hr, and further small amounts thereafter (3·4% in 24–38 hr and 4·1% in the 48–96 hr periods after which excreted radioactivity was too small to be measured accurately).

The decline in the rate of excretion of urinary radioactivity 2 hr after the standard test may not have been due to a decrease in the absorption of oral thiamine but may have followed a fall in the blood level of the flushing dose due to the rapid excretion. Consequently, radioactive thiamine (5·0 μCi) was incorporated in a 200 mg intravenous injection on non-radioactive thiamine to investigate the duration of the flushing dose. This showed that 4·5% (9·0 mg) of the intravenous dose was still being excreted during the 4–5 hr period. Similarly, varying the time of the flushing dose or maintaining a high blood level of non-radioactive thiamine during the first 16 hr by repeated injections had a negligible effect on the total urinary recovery

(Table 2). When the standard test alone was repeated in four subjects, the following results were obtained: 46·0, 38·4; 49·0, 54·0; 58·4, 54·7; 60·0, 62·7%.

Eighty-eight per cent of the intravenous dose was recovered during the first 24 hr and 90% after the radioactive thiamine was given intramuscularly to the same subject (Fig. 2). Another subject excreted 93% of an intravenous dose in 24 hr. Consequently, it seems that practically

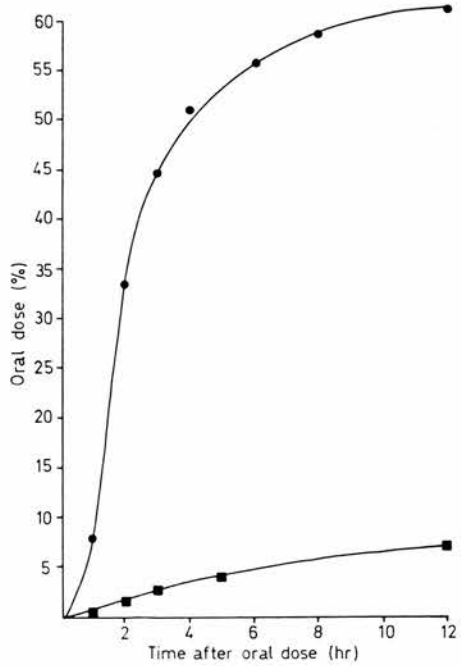

FIG. 1. Cumulative urinary excretion of radioactivity after 1·0 mg [^{35}S]thiamine orally and 200 mg non-radioactive thiamine intravenously in a control subject (●) and in a patient with primary malabsorptive disease (■).

all of the oral dose absorbed, once it reaches the blood stream, will probably be excreted during the first 24 hr, under the conditions of the standard test.

Studies on patients

Primary malabsorptive disease. The excretion of eight patients with primary malabsorptive disease given 300 mg intravenously 48 hr before the standard test was 26·7 (SEM ± 5·11) which was significantly less than the control group ($t = 3{\cdot}504$, $P < 0{\cdot}01$). When the 300 mg 'loading' dose was omitted there was no decrease in the recovery of urinary radioactivity 28·1% (SEM ±4·75). The difference between these patients and the controls was not due to a decreased ability to excrete thiamine as a patient was able to excrete an intravenous dose normally. The cumulative excretion of radioactivity in a 'loaded' untreated patient showed delay in excretion and marked reduction in the total excreted 9·4% although maximal excretion occurred within the first 6 hr (Fig. 1). Additional flushing doses given to three malabsorptive patients during the

0–24 hr period and to two others during the 24–48 hr period did not alter the pattern of excretion or significantly increase the total radioactivity excreted; the details of one patient are shown in Table 3.

In thirteen patients who had been treated with a gluten-free diet and replacement therapy, previous loading again had no effect on the excretion of radioactivity (Table 4). The previously

TABLE 2. Effect of varying the time of the initial flushing dose and giving additional intravenous flushing doses

Subject	Test	[^{35}S]thiamine urinary excretion (%)
1	First	49·0
	Second	53·0
2	First	49·0
	Second	49·0
2	First	41·7
	Second	41·2
4	First	75·0
	Second	80·0
5	First	58·5
	Second	58·0
6	First	59·9
	Second	59·0

Subjects were given two tests: First* and Second† and received 300 mg non-radioactive thiamine intravenously 48 hr before each test.

* First test, 1·0 mg radioactive thiamine orally and 200 mg non-radioactive thiamine flushing dose intravenously at the same time.

† Second test, Subjects 1–3 same as first test with additional 100 mg flushing doses at 2, 6 and 12 hours after oral dose; Subject 4, same as first test with additional 200 mg at 4 hr; Subject 5, received 200 mg i.m. only at time of oral dose; Subject 6, no flushing dose at time of oral dose but 200 mg i.v. 1 hr after.

'loaded', treated patients did not differ from the control group ($t = 2{\cdot}012$, $0{\cdot}5 < P < 0{\cdot}1$) but differed significantly from the untreated patients ($t = 2{\cdot}231$, $0{\cdot}02 < P < 0{\cdot}05$).

Miscellaneous conditions. The results of eight patients with gastroenterostomies are shown in Fig. 3 and there is no evidence of abnormal absorption ($t = 0{\cdot}395$, $P < 0{\cdot}5$).

One patient with a partial gastrectomy excreted 71·6% of the oral dose while another excreted only 3·8%; this second patient had an abnormal villous pattern which may have been associated with altered gastric function. He excreted 89% in the first 24 hr when the same dose was given intravenously. A patient who had had a sub-tota gastrectomy 117 years previously excreted 65·5%.

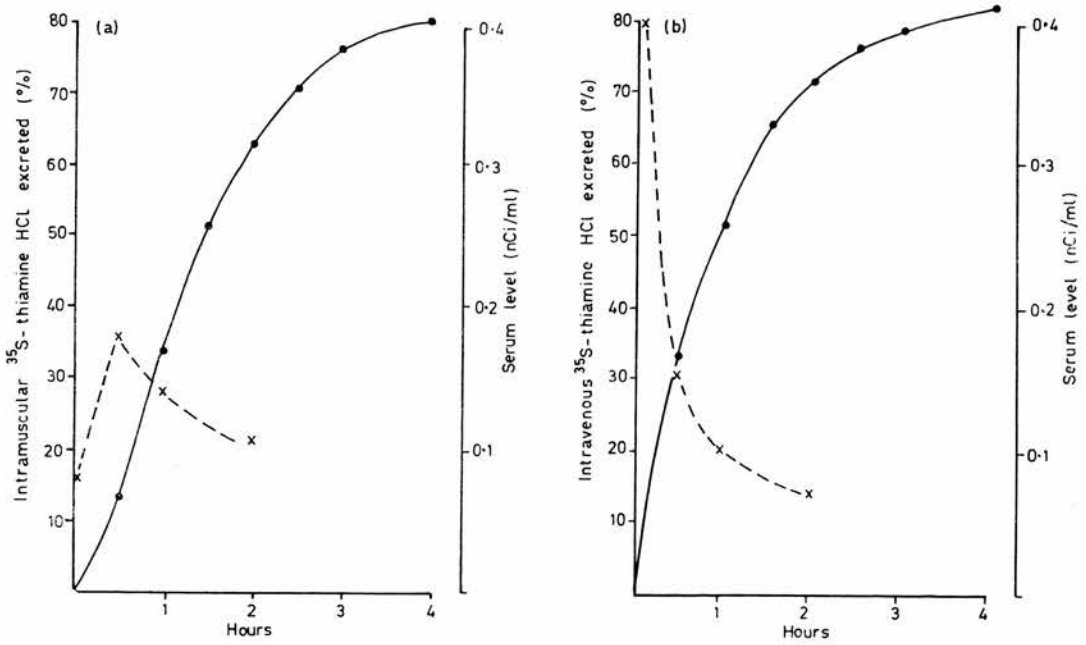

FIG. 2. The radioactivity in serum (×) and urine (●) after administration of radioactive thiamine (a) intramuscularly, and (b) intravenously, on different occasions to the same control subject. 200 mg non-radioactive thiamine were given intravenously along with the radioactive thiamine.

TABLE 3. Effect of additional flushing doses in a patient with untreated primary malabsorptive disease

Time (hr)	Urinary radioactivity after standard test	Time of additional 100 mg i.v. flushing doses of thiamine (hr)	Urinary radio-activity after additional flushing
0–2	0·3		0·3
2–4	7·4		7·0
		4	
4–6	4·8		6·1
		6	
6–12	6·4		10·9
		12	
12–24	6·3		5·0
Total	25·2		29·3

The patient was given 300 mg thiamine intravenously 48 hr before each test.

TABLE 4. Thiamine absorption in patients with primary malabsorptive disease

Subjects	No. of subjects	Before loading	After loading
Untreated	8	28·1 ± 4·75	26·7 ± 5·11
Treated	13	36·9 ± 4·33	40·8 ± 3·84

Standard test repeated in the same subject after giving 300 mg thiamine intravenously 48 hr before the second test.

The results are expressed as the mean ± SEM (*n*).

Loading was achieved as before (see Table 1).

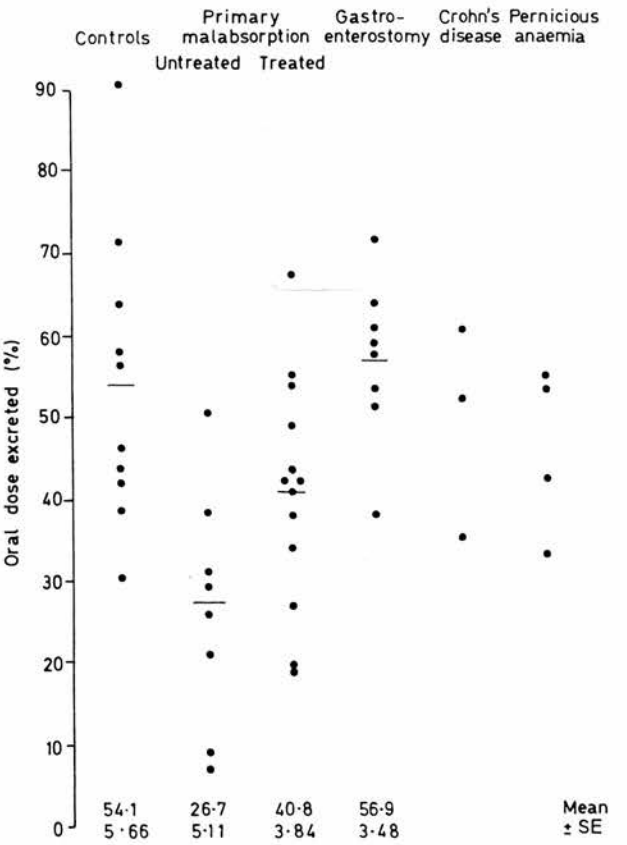

FIG. 3. Thiamine absorption in various clinical states.

Three patients with Crohn's disease involving the terminal ileum, who had evidence of malabsorption of vitamin B_{12} and a reduced serum vitamin B_{12} level, gave normal results (Fig. 3). Four patients with untreated pernicious anaemia also showed normal results.

DISCUSSION

These investigations indicate that radioactive thiamine can be used to study the absorption of this vitamin in man. Analysis of small intestinal juice and extraction of the radioactivity present in the urine shows that, during the period when maximal absorption is taking place, little breakdown of thiamine occurs in the small intestine and that most of the radioactivity obtained in the urine is thiamine which was unchanged prior to absorption.

When a relatively large injection of non-radioactive thiamine is given intravenously, the percentage excretion of radioactivity rises from about 6·0% to over 50·0%. Since the non-radioactive parenteral thiamine promotes the urinary excretion of the labelled vitamin, it would seem that the radioactive thiamine behaves similarly to the non-radioactive thiamine during its passage through the body. Increasing the flushing dose to 300 mg thiamine does not produce any further increase in the percentage urinary excretion of radioactive thiamine (Table 1, $P<0{\cdot}5$) and it would therefore seem unlikely that the flushing dose is altering the normal physiological activity of the intestine.

If an absorption test is to be sensitive to physiological amounts of the test substance, the size of the test dose must be large enough to show that the subject is capable of absorbing his minimum daily requirement during the 24 hr but must not exceed the reserve capacity of the intestine in the control subject. The minimum daily requirement of thiamine for the adult human has been estimated at between 0·23 and 0·66 mg/1000 cal (Williams *et al.*, 1942; Oldham *et al.*, 1944; Daum, Tuttle & Wilson, 1949; Dick *et al.*, 1958; Ziporin *et al.*, 1965). The work of Morrison & Campbell (1960), Friedmann *et al.* (1948) and Schultz, Light & Frey (1938) suggests that little further absorption occurs when the dose of thiamine exceeds 4–5 mg. Therefore 1·0 mg was chosen as a reasonable oral dose.

Although one control patient excreted 90·7% of the oral dose within the first 24 hr, the mean excretion for the group was only 54·1% (SEM $\pm$ 5·66) and prolonging the urine collection for 9 days in one subject added only a further 7·5%. The total thiamine content of the human body has been estimated to be 30 mg (Takeda, 1947). An attempt was made to reduce any exchange with the body stores by previous loading of the patient and by the provision of additional flushing doses each of 200 mg of non-radioactive thiamine to dilute the absorbed radioactive thiamine. However, giving additional flushing doses, or variation in time or route of administration of the flushing dose, did not significantly increase the total 24 hr excretion of activity nor alter the pattern of excretion. Also Yano (1958) recovered approximately 0·5 mg of a 2·0 mg oral dose of thiamine from the stool. Consequently, it would seem that most of the oral dose absorbed is excreted during the first 24 hr, under the conditions of the test. Haugen (1961) showed that excessive amounts of thiamine are rapidly excreted by the kidney and in the present investigation there was no evidence that at any time the kidneys were unable to excrete the load presented to them.

In control subjects the major period of absorption was in the first 2 hr after the oral dose (Fig. 1). The work of Middleton & Grice (1964) in the rat indicates that the site of maximum absorption for thiamine is in the duodenum and upper small intestine, and this agrees with the

findings of Polin *et al.* (1964) in the chick. Three patients with Crohn's disease involving the lower small intestine and showing impaired absorption of vitamin B_{12} excreted normal amounts of radioactivity after the standard test for thiamine, suggesting that absorption of vitamin B_1 in the human is mainly in the upper small intestine.

Wang & Harris (1939) and Brummer & Markkanen (1960) measured the daily urinary excretion of dietary thiamine in achlorhydric subjects and found evidence of reduced excretion indicating depletion which might have been secondary to reduced absorption. During the present investigation, four patients with pernicious anaemia and three with histamine-fast achlorhydria following gastric surgery, were all found to excrete normal amounts of radioactivity. If absorption is grossly impaired, malabsorption may become apparent when the test dose is small. A larger oral dose, however, may result in a greater difference between normals and patients or may demonstrate impairment which was not obvious with the smaller dose as occurs with xylose absorption. Consequently, the results in alchorhydria are not necessarily incompatible with the observations of Wang & Harris (1939) and Brummer & Markkanen (1960) although there may be other factors producing depletion in their patients.

The paired *t*-test showed no significant difference between malabsorptive patients who had been previously loaded and those who had not, either before, or after, treatment. It was also shown that the mean of the untreated malabsorptive patients with previous 'loading' was significantly less than the treated patients. However, the mean of the treated malabsorptive patients, who were not given 300 mg 48 hr before treatment, was low. Consequently, further statistical tests were applied. Seven of the eight differences in the untreated patients were positive but the result of the Wilcoxon's sign test (Spiegel, 1956) confirmed the *t*-test findings. Similarly, in control subjects, previous 'loading' did not significantly alter the result. Consequently, the average of the result before and after 'loading' was taken in each malabsorptive patient and the resulting two groups compared with the control group considered as twenty independent observations. A one-way analysis of variance on these three groups was found to be highly significant, confirming the difference between the untreated and control groups. $P[F \pm 5{\cdot}21, \text{d.f. } (2,38)] = 0{\cdot}99$. The one-tailed *t*-test applied to the average of the treated and untreated malabsorptive patients, gave $t = 1{\cdot}8$ with 19 d.f. and this was significant at the 5% level. There was no correlation between the ability of the malabsorptive patients to absorb thiamine and other intestinal absorption tests or with the severity of the changes shown by jejunal biopsy. The results in these patients illustrate again the variation in the ability of individual patients to absorb different substances during the acute phase of the disease.

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Thiamine Absorption in Old Age

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Introduction

Evidence of thiamine deficiency has recently been reported in elderly subjects (*Brin et al.*, 1964; *Brin et al.*, 1965; *Griffiths et al.*, 1965). The role of intestinal absorption in determining the nutritional status, however, has not been extensively studied in old age. This problem is of particular interest because of reports of structural changes in the small intestine in later life (*Lascalea*, 1959; *Andrew*, 1961; *Suntzeff* and *Angeletti*, 1961), and the demonstration by *Fry et al.* (1961) of a reduced rate of cell production with aging in the jejunal crypts of mice.

In old age too, there have been reports of impaired absorption of fat (*Becker et al.*, 1950), amino acids (*Wild et al.*, 1953), galactose (*Meyer et al.*, 1943) and of certain vitamins – vitamin A (*Yiengst* and *Shock*, 1949) and vitamin B_{12} (*Chow*, 1954; *Glass et al.*, 1955). However, *Chinn et al.* (1956) found no significant difference in the rate or the extent of protein digestion and absorption in old age and the findings of impaired absorption of vitamin B_{12} have not been confirmed by subsequent work (*Swenseid et al.*, 1954; *Chow et al.*, 1956; *Tauber et al.*, 1957; *Hyams*, 1964).

Rajsky and *Newman* (1943) measuring the urinary excretion of thiamine in twenty-two elderly subjects found that a larger dose was required to produce a constant output than in younger subjects and concluded that absorption was poorer in the older individual. The work of *Draper* (1958) in the rat showed that the absorption of an oral dose of radiothiamine decreased substantially beyond the age of 20 months. *Kirk* and *Chieffi* (1951) measuring faecal thiamine in man, however, found little change in percentage absorption with increasing age at the dose level used.

It is possible to study vitamin B_1 absorption in man using sulphur-labelled thiamine (*Thomson*, 1966) and in the present study its absorption is compared in 24 elderly patients and 21 younger subjects.

Method and Patients

Vitamin B_1 absorption was measured using sulphur-labelled thiamine hydrochloride as described by *Thomson* (1966) modified by giving two additional oral doses. ^{35}S-thiamine, specific activity 158.2 mc/g was shown to be radiochemically pure when tested in the following chromatographic systems; N-propanol/water/IM acetate buffer pH 5 (70:20:10) : N-butanol/acetic acid/water (40:10:50), sec-butanol/water and isopropanol/hydrochloric acid/water (130:33:37). The radioactive material was diluted with non-radioactive thiamine so that each test dose contained 1.0 mg, 5.0 mg or 20.0 mg of thiamine and 5 μc of radioactivity dissolved in 20 ml of water. The test dose was given orally after an overnight fast and a parenteral injection of 200 mg of non-radioactive thiamine hydrochloride was given immediately before the oral dose. Urine collections were removed after 5 h, 12 h, 24 h and 48 h and counted in a Packard Tricarb liquid scintillation counter.

Adequacy of the flushing dose when used with larger oral doses of radioactive thiamine was tested. Twenty-four control subjects were given either 1.0 mg, 5.0 mg or 20.0 mg of radioactive thiamine orally together with 200 mg of non-radioactive thiamine intravenously. The results were compared with those obtained in the same subject, given the same test as before, but with additional 100 mg intravenous injections of non-radioactive thiamine at 4 h, 9 h, 12 h and 24 h after the oral dose. Urine was collected for 72 h after each test (Table I). A one-way analysis of variance computed on the difference between the two tests showed that giving extra flushing doses did not significantly alter the excretion. P [$F \pm 0.704$; d.f. (2,22)] = 0.99. Similar results were found when the two tests were given to three subjects over the age of 80 years using 20.0 mg oral dose; 23.5%, 24.2%; 21.0%, 22.7%; 17.7%, 16.3%. The giving of additional flushing doses did not significantly increase the total excretion of activity nor alter the pattern of excretion.

Table I

Effect of additional intravenous flushing doses in controls
Receiving 1.0 mg, 5.0 mg, or 20.0 mg of radioactive thiamine orally

Oral dose radioactive thiamine mg	Urinary excretion of radioactivity % oral dose*	
	First test**	Second test***
1.0	44.7 ± 5.75 (8)	45.4 ± 5.65 (8)
5.0	33.0 ± 2.68 (8)	35.3 ± 2.15 (8)
20.0	25.9 ± 2.62 (8)	25.5 ± 1.68 (8)

* The results are expressed as the mean ± S.E. (n).

** *First test.* 1.0 mg, 5.0 mg, or 20.0 mg of radioactive thiamine orally and 200 mg of non-radioactive thiamine flushing dose intravenously at the same time.

*** *Second test.* Same as for first test with additional 100 mg flushing doses at 4, 9, 12 and 24 h after oral dose.
Urine was collected for 72 h.

Subjects. Two groups of convalescent hospital in-patients who were free from haematological, gastro-intestinal, endocrine or malignant disease, were studied:

Group I consisted of 24 subjects aged 76–90 years (mean 82.1 years). Group II consisted of 21 younger subjects aged from 28–56 years (mean 48.9 years). Fifteen members of the older group were given a test at each of the three oral dose levels, i.e. 1.0 mg, 5.0 mg and 20.0 mg of thiamine. The order in which the tests were performed was randomised. The remaining nine were given one test only. Ethical reasons prevented more than two tests being carried out on any individual in the younger group. The dose to be omitted was again determined from random number tables (*Documenta Geigy*, 1965).

Results

The results obtained are summarised in Table II. In Fig. 1–3 the percentage excretion of radioactivity of both the younger and the older groups, at each dose level, have been plotted against age.

Table II

Thiamine absorption in younger and older groups at three oral dose levels

Oral dose radioactive thiamine mg	Urinary excretion of radioactivity % oral dose*	
	Older	Younger
1.0	53.1 ± 3.03 (21)	51.8 ± 2.97 (16)
5.0	33.4 ± 2.14 (17)	40.8 ± 4.71 (12)
20.0	21.2 ± 1.23 (18)	25.8 ± 3.69 (14)

* The results are expressed as the mean ± S.E. (n). Urine was collected for 48 h. Subjects were given 200 mg of non-radioactive thiamine flushing dose intravenously at the time of the oral dose.

A regression analysis was performed at each of the dose levels but no correlation between age and the percentage excretion of radioactivity could be demonstrated. [At 1.0 mg, oral dose/age $t = \pm 0.002$ (35 d.f.) $p > 0.5$; 5.0 mg $t = \pm 0.687$ (27 d.f.) $p = 0.5$; 20.0 mg $t = \pm 0.673$ (30 d.f.) $p > 0.5$].

Discussion

The results agree with those of *Kirk* and *Chieffi* (1951). They measured faecal thiamine using a chemical method (*Hennessy* and *Cerecedo*, 1939; *Friedmann* and *Kmieciak*, 1943) before, during and after a 5.0 mg daily oral supplement but found little change in the percentage absorption with increasing age. The minimum daily requirement of thiamine for the young adult has been estimated at

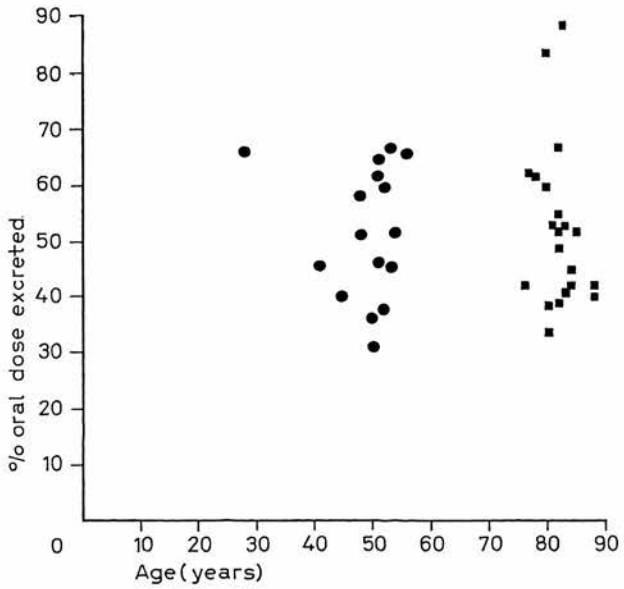

Fig. 1. Excretion of 1.0 mg oral dose ^{35}S-thiamine at different ages.
● Younger group.
■ Older group.

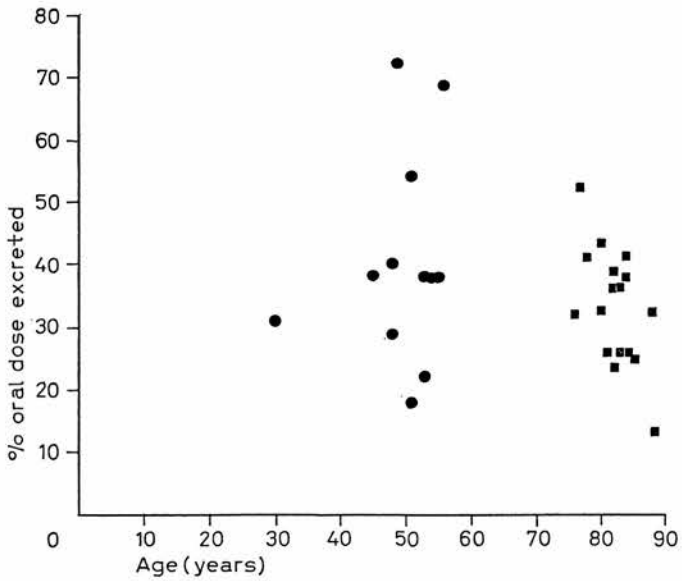

Fig. 2. Excretion of 5.0 mg oral dose ^{35}S-thiamine at different ages.
● Younger group.
■ Older group.

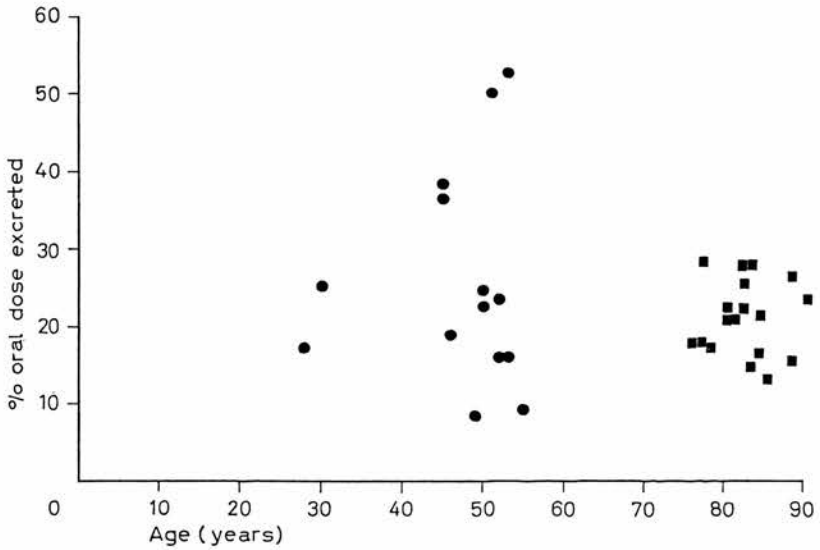

Fig. 3. Excretion of 20.0 mg oral dose ^{35}S-thiamine at different ages.

● Younger group.
■ Older group.

between 0.23 mg/1000 cal and 0.66 mg/1000 cal (*Williams et al.*, 1942; *Oldham et al.*, 1944; *Daum et al.*, 1949; *Dick et al.*, 1958; *Ziporin et al.*, 1965) and the daily allowance suggested by the National Research Council is 1.3 mg per day (*National Research Council Publication*, 1958). Evidence suggests that for the rat (*Mills*, 1948) and for man (*Oldham*, 1962) this requirement may be increased in old age. However, both at a physiological level and at very much larger doses, no difference in absorption has been found between the two groups in this present study. This is not to say that deficiency may not occur due to inadequate intake of thiamine. Poor diets may result from prejudice about food or follow mental deterioration, or heat labile nutrients may be destroyed by prolonged cooking. Striking deterioration in health and nutrition has been reported in the late seventies when both weight loss and reduction in calorie intake often occur (*Exton-Smith* and *Stanton*, 1965).

Because of the criteria of selection, no comments can be made on the absorption of patients with haematological, gastro-intestinal, endocrine or malignant disease. Nor were any subjects studied who had advanced protein malnutrition in which severe intestinal atrophy has been observed (*Passmore*, 1947–1948; *Gillman* and

Gillman, 1951; *Brock*, 1961; *Deo* and *Ramalingaswami*, 1964). However, the findings do suggest that there is not an inevitable decline with age in the ability of the intestine to absorb vitamin B_1 and that deficiency in most elderly people need not arise provided that adequate amounts are present in the diet.

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Summary

The ability of 24 elderly subjects (mean age 82.1 years) to absorb thiamine has been investigated using three oral doses 1.0 mg, 5.0 mg and 20.0 mg. The results were compared with those in 21 younger subjects (mean age 48.9 years) but no evidence of impaired absorption was found. No inevitable decline with age in the ability of the intestine to absorb vitamin B_1 seems to occur and deficiency in most elderly people probably need not arise provided they are receiving an adequate diet.

Zusammenfassung

Bei 24 alten Personen (82,1 Jahre durchschnittlich) wurde die Thiaminresorption bei Einnahme von 1,0 mg, 5,0 mg und 20,0 mg oral untersucht. Die Resultate wurden mit den von 21 jüngern Personen verglichen (38,9 Jahre durchschnittlich), aber es konnte kein Unterschied in der Absorption festgestellt werden. Es scheint, daß im Alter keine unvermeidliche Abnahme der Vitamin B_1-Resorption im Darm vorkommt; der Mangel bei den meisten alten Leuten müßte bei entsprechender Diät nicht vorkommen.

Résumé

Les auteurs ont testé le pouvoir d'absorption de la Vitamine B_1 chez 24 sujets âgés (âge moyen de 82,1 ans). Les résultats furent comparés à ceux enregistrés chez 21 sujets plus jeunes (âge moyen 48,9 ans). Aucune altération évidente de la capacité d'absorption n'a pu être objectivée. Les carences chez le vieillard semblent donc pouvoir être facilement évitées à condition que celui-ci bénéficie d'un régime équilibré.

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